ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 19 **March 1990** **Number 1**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1990: Volume 19 (4 issues).
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTENATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 19

1990

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 19 (1), pp. 3—7 (1990)

CHANGES OF ELDERBERY *(SAMBUCUS NIGRA)* PIGMENTS DURING THE PRODUCTION OF PIGMENT CONCENTRATES

M. Drdák and P. Daucik

Department of Chemistry and Technology of Saccharides and Foods, Faculty of Chemical Technology, Slovak Technical University, 812 57 Bratislava. Czechoslovakia

(Received: 4 November 1988; accepted: 2 May 1989)

The paper is a study on the observation of the individual pigments contained in elderberries *(Sambucus nigra)* during the production process of pigment concentrate in accordance with a patented method. High pressure liquid chromatography has been used for the distribution of the pigments. In the course of the fermentation and pigment concentration processes, the representation of the individual pigments (cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside) has been followed. It was found that the relative representation of the individual pigments undergoes changes in the process course, with the cyanidin-3-sambubioside found as the most stable antho-cyanin-based pigment in elderberries.

Keywords: anthocyanin-based pigments, changes of pigments during fermentation, elderberry *(Sambucus nigra)*, HPLC

The decreasing trend to use synthetic dyes for colouring of food articles has intensified the interest to utilize new natural source of colouring substances. One of the rich sources of anthocyanin-based pigments is represented by the fruit of elderberry *(Sambucus nigra)*. Previous papers have referred to the properties and determination of anthocyanins in elderberries (Drdák et al., 1983a), to the process of preparation of colouring substance concentrates from the berries (Drdák et al., 1983b; Drdák et al., 1985), to the stability of the pigments in individual stages of the process (Drdák & Hayden, 1983c) as well as in solution containing increased sugar and alcohol concentrations (Drdák & Hayden, 1983d); Drdák & Kukučková, 1984; Drdák et al., 1985), and to the stability problem of isolated pigment fractions (Dydák & Voysová, 1984). However, the proper process control and application of the pigment concentrate require the knowledge of the influence of individual operations upon the composition and representation of individual pigments, which constitute the topic of the present paper.

1. Material and methods

The elderberry juice was prepared by pressing the berries and used for the preparation of the pigment concentrate in compliance with a patented process (DRDÁK et al., 1985), involving pasteurization of the juice at 90–95 °C, its fermentation using *Saccharomyces oviformis* Tokaj 75 D yeast cells (48 h, 28 °C) as well as clarification and vacuum concentration (45–50 °C).

Recommended processes (BRONNUM-HANSEN & HANSEN, 1983) were applied for the separation, distribution and determination of the relative amounts of anthocyanins in the fresh juice, fermented juice and in the pigment concentrate. In the process of anthocyanin separation, a highly efficient VARIAN 8500 liquid chromatograph has been used in conjunction with a UV-VIS VARISCAN 634 D spectrophotometer, an A-25 recorder and a high-pressure injection system (stop-flow).

Analytic conditions were as follows: Solvent A, 0.05 mol H_3PO_4 in water; pH = 1.8; Solvent B: 50% tetrahydrofurane in water, flow rate: 0.5 cm^3 min^{-1}, column: SEPHARON SIX-C 18 (150 mm height, internal diameter 3.2 mm), gradient: start – 15% of Solvent B, increased by 1% min^{-1}; termination – 45% of Solvent B; pressure 12 MPa; wavelength 510 nm.

2. Results and discussion

Our work has been concentrated upon the comparison of individual anthocyanin fractions in the fresh juice, in the pasteurized juice, and in the concentrate, respectively. The use of standard HPLC conditions of distribution of the elderberry fruit pigment as recommended (BRONNUM-HANSEN & HANSEN, 1983) guarantees the elution process of the individual pigments from the column in specified order as shown in the reference cited. Measurements of the absorption spectra and comparison of the R_f values along with the determination of the molar absorption coefficients (NYBOM, 1968) were considered as sufficiently conclusive for the following representation and occurrence of the anthocyanin pigments. The following figures show typical HPLC recordings for the elderberry pigments in both fresh and pasteurized juice as well as in the pigment concentrate. Table shows data related to the occurrence of individual pigments and their relative distribution. The total amount of pigments in the juice and in the concentrate was determined by the pH-differential method (FÜLEKI & FRANCIS, 1968), with the mean absorption coefficient determined with respect to the original distribution of the anthocyanin pigments in the elderberry fruit. The table shows that 3,5-diglucosides represent less than 16% of the total anthocyanins present, with cyanidin-3-sambubioside-5-glucoside being present in much larger amounts than cyanidin-3,5-diglucoside. Mo-

Table 1

The relative representation (%) of anthocyanins in the fresh fermented juice and in the pigment concentrate produced from elderberries

Identification	Fresh juice			Fermented juice			Pigment concentrate		
	$\bar{x}$	s	σ	$\bar{x}$	s	σ	$\bar{x}$	s	σ
Cy-3-Sb-5-Gl	10.17	0.15	0.13	14.08	0.16	0.13	10.75	0.15	0.11
Cy-3-Gl-5-Gl	2.04	0.11	0.10	1.81	0.12	0.10	1.31	0.12	0.10
Cy-3-Sb	55.23	0.28	0.25	68.95	0.33	0.30	68.82	0.35	0.31
Cy-3-Gl	32.56	0.23	0.21	15.16	0.17	0.15	19.12	0.18	0.15

$$\bar{x} = \frac{\Sigma x_i}{n} \qquad s = \left[\frac{\Sigma(x_i - \bar{x})^2}{n-1}\right]^{1/2} \qquad \sigma = \left[\frac{\Sigma(x_i - \bar{x})^2}{n(n-1)}\right]^{1/2}$$

noglucosides are presented by cyanidin-3-sambubioside and cyanidin-3-glucoside, with the former compound dominating.

The results have enabled to draw conclusions related to the stability of individual pigments as followed by HPLC. In the course of pasteurization and fermentation, the cyanidine-3-glucoside has shown the lowest stability, with its relative percentage dropping from the original 32.56 to 15.16%. Less decrəase has been experienced in the case of cyanidin-3,5-diglucoside, whəreas

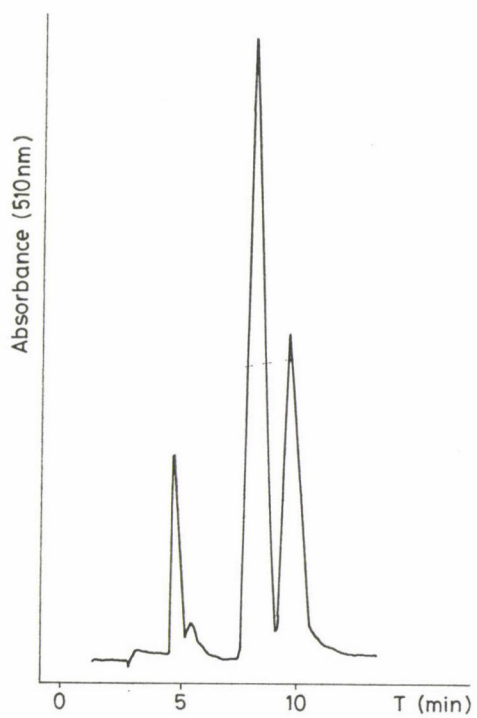


Fig. 1. Chromatographic recording of the fresh juice

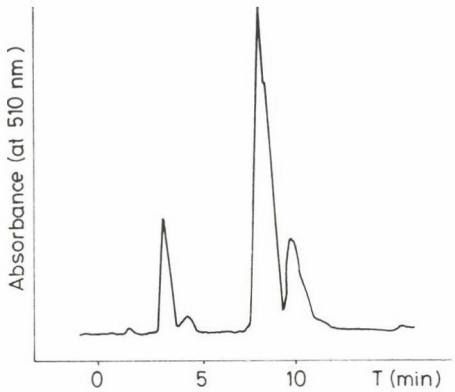


Fig. 2. Chromatographic recording of the fermented juice

cyanidin-3-sambubioside-5-glucoside, and cyanidin-3-sambubioside have during the fermentation process demonstrated a comparative grade of stability against cyanidin-3,5-diglucoside and cyanidine-3-glucoside. However, their decomposition and/or stability, respectively, could not be based upon the method used, attributed unanimously to the possible influence of the environment or of the fermetation process. For completeness sake it must be pointed out that the original process of elderberry pigment concentrate, with respect to the 5% inoculum, optimal temperature, and the use of deep-fermenting yeast cells was based upon the optimum duration (24 h) of the process of

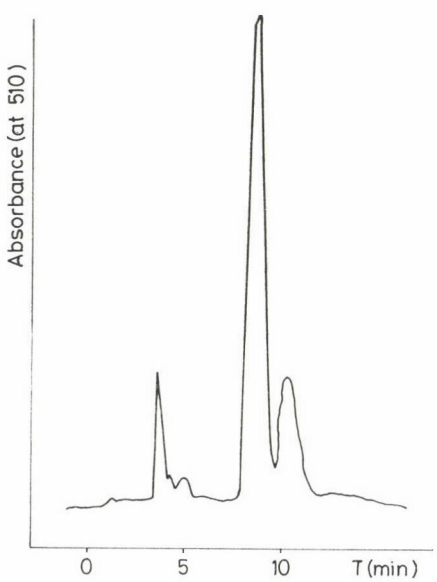


Fig. 3. Chromatographic recording of the pigment concentrate

fermentation in order to maintain maximum amounts of pigments and to obtain the necessary decrease in the concentration of fermentable sugars. The prolonged process of fermentation was expressed by the lower relative representation of cyanidin-3-glucoside resulting in a lower pigment concentration in the final product. In spite of the maximum pH-adjustment, the prolonged fermentation process resulted in an increased anthocyanin loss.

During concentration (by evaporation) of the clarified fermented juice, highest pigment losses were found in the case of diglucosides, i.e. cyanidine-sambubioside-5-glucoside, while cyanidin-3,5-diglucoside has in this stage of concentrate preparation shown a high degree of stability. From the aspect of evaluation of the entire process of production of the natural pigment concentrate it was found that the most stable anthocyanin pigment in elderberries during fermentation and production of the concentrate has been the cyanidin-3-sambubioside.

Literature

BRONNUM-HANSEN, L. K. & HANSEN, ST. H. (1983): High performance liquid chromatographic separation of anthocyanins of *Sambucus nigra* I. *J. Chromat., 262,* 385–392.

DRDÁK, M., GREIF, G. & FARKAŠOVÁ, I. (1983a): Farbivá bazy čiernej *(Sambucus nigra)* I. Vlastnosti a stanovenie farbív bazy čiernej. (Elderberry *(Sambucus nigra)* pigments. I. Characteristics and determination of elderberry pigments.) *Bull. potrav. výskumu, 22,* 21–28.

DRDÁK, M., MALÍK, F., ŠILHÁROVÁ, A. & GREIF, G. (1983b): Farbivá bazy čiernej *(Sambucus nigra)* II. Postup spracovania bazy čiernej na koncentrát farbív. (Elderberry *(Sambucus nigra)* pigments. II. Method of processing elderberries to pigments concentrate.) *Bull. potrav. výskumu, 22,* 29–35.

DRDÁK, M. & HAYDEN, I. (1983c): Farbivá bazy čiernej *(Sambucus nigra)* III. Porovnanie stability farbív v surovej, pasterizovanej a fermentovanej šťave. (Elderberry *(Sambucus nigra)* pigments. III. Comparison of pigment's stability in raw, pasteurized and fermented juice.) *Bull. potrav. výskumu, 22,* 149–155.

DRDÁK, M. & HAYDEN, I. (1983d): Farbivá bazy čiernej *(Sambucus nigra)* IV. Stability farbív v roztokoch so zvýšenou koncentráciou cukru. (Elderberry *(Sambucus nigra)* pigments. IV. Stability of pigments in solutions with an increased concentration of sugar.) *Bull. potrav. výskumu, 22,* 157–165.

DRDÁK, M. & KUKUČKOVÁ, B. (1984): Farbivá bazy čiernej V. Stabilita farbív v roztokoch so zvýšenou koncentráciou etylalkoholu. (Elderberry *(Sambucus nigra)* pigments. V. Stability of pigments in solutions with an increased concentration of etylalcohol.) *Bull. potrav. výskumu, 23,* 131–136.

DRDÁK, M. & VORSOVÁ, R. (1984): Farbivá bazy čiernej *(Sambucus nigra)* VI. Izolácia frakcií farbív a ich vlastnosti. (Elderberry *(Sambucus nigra)* pigments. VI. Isolation of pigment's fractions and their properties.) *Bull. potrav. výskumu, 23,* 225–233.

DRDÁK, M., DANDÁR, A. & KUKUČKOVÁ, B. (1985): Zum Studium der Eigenschaften der Farbstoffe in schwarzem Holunder *(Sambucus nigra). Ind. Obst Gemüseverw., 70,* 355–358.

DRDÁK, M., MALÍK, F., GREIF, G. & ŠILHÁROVÁ, A. (1985) 9 Spôsob prípravy farbiaceho preparátu z plodovej bazy čiernej. (Method of processing elderberries to pigments preparation.) CSSR Patent 225 530.

FÜLEKI, T. & FRANCIS, F. I. (1968): Quantitative methods for anthocyanins 2. Determination of total anthocyanin and degradation of total anthocyanin and degradation index for Cranberry juice. *J. Fd. Sci., 33,* 78–83.

NYBOM, N. (1968): Cellulose thin layers for anthocyanin analysis with special reference to the anthocyanins of the black raspberries. *J. Chromat., 38,* 382–387.

Acta Alimentaria, Vol. 19 (1), pp. 9—17 (1990)

INVESTIGATION ON CHILLING SENSITIVITY OF FRUITS AND VEGETABLES USING ARRHENIUS PLOTS

T. MURATA

Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka. Japan

(Received: 10 January 1989; accepted: 18 April 1989)

A possibility to use the Arrhenius plots for the determination of the critical temperatures for chilling injury of fruits and vegetables was investigated statistically. A relatively close relationship between the temperatures for break points in the Arrhenius plots of the rate of potassium ion from the tissues (discs) and the critical temperature for chilling injury of several varieties of vegetables was found ($r^2 = +0.310$, significant at 5%). In the case of Arrhenius plots of respiratory rate of several vegetables, a coefficient of correlation (r^2) was +0.151 that was not significant, because of too small degree of freedom. A significantly close relationship between the temperatures for break points in the plots of respiratory rate and the critical temperatures for chilling injury was observed in some varieties of citrus fruits ($r^2 = +0.674$, significant at $P \geq 1\%$ probability level).

From these results, it can be cocluded that the temperatures for break points in the Arrhenius plots are useful for determination of the critical temperatures for chilling injury of fruits and vegetables under certain restrictions.

Keywords: chilling sensitivity, chilling injury, fruits and vegetables, Arrhenius plots

It is well known that keeping fruits and vegetables chilled all the way from farmers to consumers is an effective way of maintaining their quality and nutritional value. However most fruits and vegetables originating from tropical and subtropical regions are chilling sensitive and easily suffer from chilling inury after holding at a temperatur lower than the critical point for a certain period (COUEY, 1982; MURATA, 1986).

Consequently, their quality and nutritional value under the chilled conditions are poor. Therefore, it is important to know what temperature is critical for chilling injury of each fruit and vegetable. There are several publications that indicate standard temperatures for chilling injury (INSTITUT INTERNATIONAL DU FROID, 1967; AMERICAN SOCIETY OF HEAT, REFRIGEGATING AND AIR-CONDITIONING ENGINEERS, 1968). However, fruits and vegetables from different climates, of different cultivars, maturities and produced under different conditions of cultivation exhibit different temperatures for the occurrence of chilling injury.

This paper deals with the possibility to use the Arrhenius equation for the determination of the critical temperatures for chilling injury of fruits and vegetables.

1. Materials and methods

The vegetable materials included as shown in Tables 1 and 2 were obtained from the experimental farm of Shizuoka University. The fruit materials included in Table 3 were obtained from local orchards in Shizuoka.

For the measurement of leakage, discs of ∅ 4–7 mm × 3–5 mm thickness, depending on the kind of material, were prepared. The freshly prepared disc (10–15 discs) were incubated in a given volume of deionized water or isotonic solution of mannitol at different temperatures, after incubation for 2 h the content of potassium which had leaked from the disc into the medium was measured using a Hitachi-207 type atomic absorption spectrophotometer (MURATA & TATSUMI, 1979; IZUMI et al., 1987). For the measurement of respiratory rate, the fruits and vegetables were incubated in a given volume of desiccators (0.8–8 l, depending on size of material) at different temperatures for a given period of time (2–5 h, depending on temperature), then the concentration of carbon dioxide in the desiccators was measured with a Hitachi-063 type gas chromatograph (MURATA et al., 1980; MURATA, 1981). The results presented were the average of seven replicates. The regression lines in the Arrhenius plots and the temperatures for break points in the plots were calculated statistically by means of a computer (NEC-PC-980). Means in the tables were separated by Duncan's multiple range test and F test on the temperatures for break points of the rate of potassium ion leakage and respiratory rate, and the critical temperatures for chilling injury.

2. Results

2.1. Arrhenius plots of the rate of potassium ion leakage from the tissue slices

Figure 1 shows the Arrhenius plots of the rate of potassium ion leakage from the discs (∅ 7 mm × pod-thickness) of snap bean cv. Master piece and the discs (∅ 4 mm × 2 mm in thickness) of sweet pepper cv. Shin-sakigake which are chilling sensitive. The regression lines in the Figure exhibit break points (discontinuity) at 7.1 °C for snap bean pods and 12.5 °C for sweet pepper.

The Arrhenius plots of rate of potassium ion leakage from the discs (∅ 7 mm × thickness of mesocarp) of tomatoes cv. Beiju at two different stages of maturity are shown in Fig. 2. The break points in the plots can be observed at 12.5 °C for mature green tomato and 7.1 °C for dark pink tomato which is more tolerant to chilling than mature green tomato.

The relationship between the temperatures for the break points in the Arrhenius plots of the rate of potassium ion leakage that may correspond

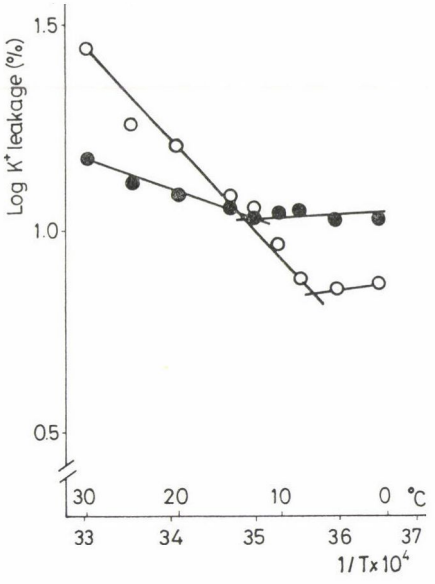


Fig. 1. Arrhenius plots of rate of potassium ion leakage from discs of tissues prepared from snap bean-pods and sweet pepper (○: snap bean cv. Master piece, ●: sweet pepper cv. Shin-sakigake)

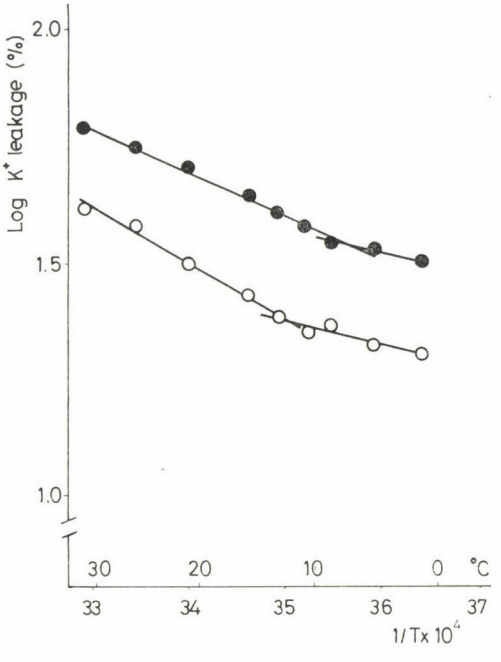


Fig. 2. Arrhenius plots of rate of potassium ion leakage from discs of tomatoes at two different stages of maturity (○: mature green, ●: dark pink)

Table 1

Relation between temperatures for break points in the Arrhenius plots of rate of K-ion leakage from tissue slices and critical temperatures for chilling injury of several vegetables

Vegetables	Temperature for break points (°C)	Critical temperature for chiling injury (°C)
Sweet pepper *Capsicum annuum* L.	12.5	6.5
Tomato cv. Beiju: mature green	12.5	9.5
dark pink *Lycopersicon esculentum* L.	7.1	5.5
Winter squash: cv. Ebisu	12.5	6.5
cv. Pepo	9.0	5.5
cv. Kogiku *Cucurbita maxima* Duch. *C. moschata* Duch.	12.5	6.5
Bitter melon (balsam pear) *Momordica charantia* L.	11.5	7.0
Sweet potato cv. Kokei No. 14. *Ipomoea batatas* Poir.	10.5	8.5
Eggplant cv. Kokuyo *Solanum melongena* L.	9.5	6.5
Water convolvulus *Ipomoea aquatica* Fork.	7.8	6.5
Snap bean cv. Master piece *Phaseolus vulgaris* L.	7.1	6.0
Cucumber: cv. Horai	6.7	6.0
cv. Pixie *Cucumis sativus* L.	5.5	5.5
Chayote *Sechium edule* Sw.	5.5	6.5
Oriental pickling melon *Cucumis melo* L.	5.2	6.0
r^2	0.310*	

* Significant at $P \geq 5\%$ probability level

to membrane permeability of the cells and the critical temperatures for chilling injury of all vegetables tested in this experiment are summarized in Table 1. In this table, the critical temperatures for chilling injury were obtained experimentally (MURATA et al., 1980; MURATA, 1981; MURATA, 1986). The coefficient of correlation (r^2) is +0.310 which is statistically significant at 5% level.

2.2. Arrhenius plots of respiratory rate of intact fruits and vegetables

Figure 3 shows the Arrhenius plots of respiratory rate of peas cv. Alaska and snap bean cv. Master piece in pods. The regression line of respiratory rate for peas which are chilling tolerant shows a nearly straight line at the temperature range from 0 °C to 20 °C. On the other hand, the line for snap beans which are chilling sensitive shows a break point at 9.6 °C.

Table 2 shows the relationship between the temperatures for break points of respiratory rate in the Arrhenius plots and the critical temperatures for chilling injury of several vegetables. There is some correlation between the temperatures for break points and the critical temperatures for chilling injury. However, the coefficient of correlation (r^2) is only $+0.151$ which is statistically not significant, and because of the heterogenous materials with too small degree of freedom.

Figure 4 shows the Arrhenius plots of respiratory rate of several varieties of citrus fruits. Break points can be observed at 13 °C for Kinukawa which is very chilling sensitive, 10.5 °C for Hassaku, 7.6 °C for Iyo, 8.8 °C for Natsudaidai and 5.5 °C for Ponkan which is relatively chilling tolerant, respectively.

Table 3 summarizes the relationship between the temperatures for break points of respiratory rate in the Arrhenius plots and the critical temperature for chilling injury of some citrus varieties. There is a close relationship between

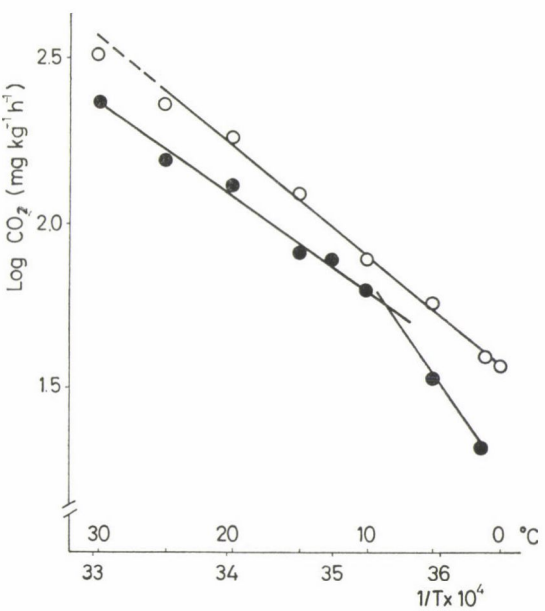


Fig. 3. Arrhenius plots of respiratory rate of pea and snap bean in pods (○: pea cv. Alaska, ●: bean cv. Master piece)

Table 2

Relation between temperature for break points of respiratory rate in the Arrhenius plots and critical temperature for chilling injury of several vegetables

Vegetables	Temperature for break points (°C)	Critical temperature for chilling injury (°C)
Water convolvulus *Ipomoea aquatica* Forsk.	13.8	6.0
Tomato cv. Beiju: mature green	12.5	9.5
dark pink *Lycopersicon esculentum* L.	10.3	5.5
Sweet potato cv. Kokei No. 14 *Ipomoea batatas* Poir.	10.0	8.5
Squash: cv. Ebisu	9.7	6.5
cv. Kogiku	10.0	6.5
cv. Yakko *Cucurbita maxima* Duch. *C. Moschata* Duch.	7.2	6.0
Snap bean cv. Master piece *Phaseolus vulgaris* L.	9.6	6.0
Eggplant cv. Kokuyo *Solanum melongena* L.	9.1	6.5
Sweet pepper *Capsicum annuum* L.	7.7	6.5
Cucumber: cv. Horai	7.2	6.0
cv. Natsuakifuchinari *Cucumis sativus* L.	6.6	6.0
r^2	0.151	

these two temperatures, and the coefficient of correlations (r^2) is +0.674 which is statistically significant at 1% level.

There have been many research reports concerning the Arrhenius plots of respiratory rate of mitochondria (LYONS & RAISON, 1970; MCGLASSON & RAISON, 1973; RAISON, 1973, YAMAKI & URITANI, 1974; KANE & MARCELLIN, 1978) and intact fruits (MINCHIN & SIMON, 1973; PURVIS, 1980; MURATA et al., 1980; MURATA, 1981; MURATA, 1982), permeability (MURATA & TATSUMI, 1979; TATSUMI & MURATA, 1981; HIRATA et al., 1987; IZUMI et al., 1987), growth (NISHIYAMA, 1975; RAISON & CHAPMAN, 1976; BREIDENBACH & WARING, 1977; BAGNAL & WOLFE, 1978). There is a controversy as to whether the abrupt discontinuity in the plots is a break or curve (WILLCOX & PATTERSON, 1979). Besides, MCMURDO and WILSON (1980) have questioned the validity of a break in the Arrhenius plots for complex physiological processes. The Arrhenius plots of respiratory rate or membrane permeability may reflect not only chilling sensitivity, but also other physiological behavior involving maturation and senescence of fruits and vegetables. However, in this experi-

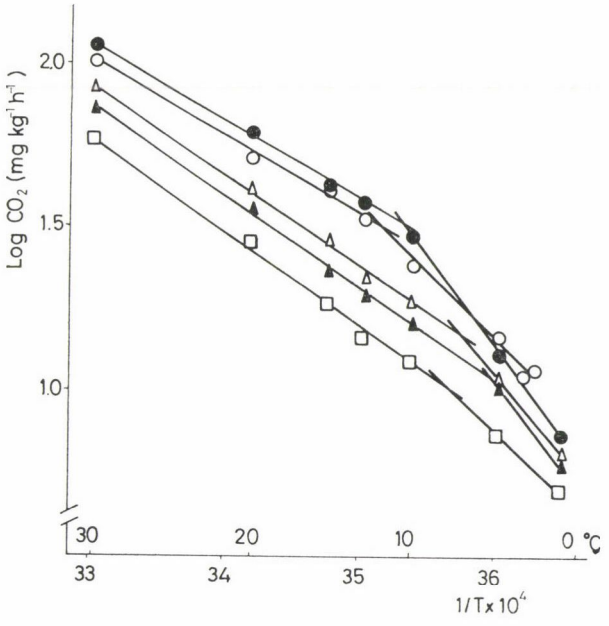


Fig. 4. Arrhenius plots of respiratory rate of some varieties of citrus fruits (○: Kinukawa, ●: Hassaku, △: Iyo, ▲: Ponkan, □: Natsudaidai)

ment, the regression lines of respiratory rate or membrane permeability of all chilling sensitive fruits and vegetables exhibit break points in the Arrhenius plots. Whereas, no break point is observed in the case of chilling tolerant fruits and vegetables. In addition, a relatively close correlation can be observed between the temperatures at these break points and the critical temperatures for chilling injury. If the comparison is especially limited to the fruits and vegetables belonging to the same variety, genus and family, there will be a significantly close correlation between these two temperatures.

3. Conclusion

It is reasonable to conclude that the breaks in the Arrhenius plots may indicate the degree of chilling sensitivity of fruits and vegetables. Under certain restrictions, it may be possible to estimate the critical temperature for chilling injury of fruits and vegetables from the temperatures at break points in the Arrhenius plots.

Table 3

Relation between temperatures for break points in the Arrhenius plots of respiratory rate and critical temperatures for chilling injury of citrus fruits

	Temperature for break points (°C)	Critical temperature for chilling injury (°C)
Kinukawa *Citrus glaberrima* hort. ex Tanaka	13.0	7.0
Hassaku *C. hassaku* hort. ex Tanaka	10.0	4.5
Navel orange *C. sinensis* Osbeck var. Brasiliensis Tanaka		
cv. Morita	9.5	4.5
cv. Shirayanagi	8.6	4.5
Natsudaidai *C. natsudaidai* Hayata cv. Kawano	8.8	4.5
Yuzu *C. junos* Sieb. ex Tanaka	8.4	2.5
Iyo *C. iyo* hort. ex Tanaka cv. Miyauchi	7.6	3.0
Sudachi *C. sudachi* hort. ex Tanaka	7.6	3.0
Ponkan *C. reticulata* Blanco	5.5	2.0
Kabosu *C. sphaerocarpa* hort. ex Shirai	4.5	4.0
Hanayu *C. hanayu* hort. ex Tanaka	4.5	3.0
Satsuma *C. unshiu* Marc.	2.5	1.0
	r^2	0.674**

** Highly significant at $P \geq 1\%$ probability level

Literature

American Society of Heat, Refrigerating and Air-Conditioning Engineers (1968): *ASHRAE Guide and data book. Application volume.* New York. pp. 637–648.

Bagnall, D. J. & Wolfe, J. A. (1978): Chilling sensitivity in plants: Do the activation energies of growth process show an abrupt change at a critical temperature? *J. exp. Bot., 29,* 1231–1242.

Breidenbach, R. W. & Waring, A. J. (1977): Response to chilling of tomato seedlings and cells in suspension culture. *Pl. Physiol., 60,* 190–192.

Couey, H. M. (1982): Chilling injury of crops of tropical and subtropical origin. *HortScience, 17,* 162–165.

HIRATA, K., CHACHIN, K. & IWATA, T. (1987): Changes of K^+ leakage, free amino acid contents and phenylpropanoid metabolism in water convolvulus *(Ipomoea aquatica* Forsk.) with reference to the occurrence of chilling injury. *J. Jap. Soc. hort. Sci., 55,* 516–523.

INSTITUT INTERNATIONAL DU FROID (1967): *Recommended conditions for cold storage of perishable produce.* 2nd ed., Paris. pp. 30–56.

IZUMI, H., TATSUMI, Y., MURATA, T. & YOSHIDA, Y. (1987): Chilling sensitivity of several vegetables by means of Arrhenius equation. *J. Jap. Soc. Cold Preserv. Fd, 13,* 1–7.

KANE, O. & MARCELLIN, P. (1978): Incidence of ripening and chilling injury on the oxidative activity and fatty acid composition of the mitochondria from mango fruits. *Pl. Physiol., 61,* 634–638.

LYONS, J. M. & RAISON, J. K. (1970): Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Pl. Physiol., 45,* 386–389.

MCGLASSON, W. B. & RAISON, J. K. (1973): Occurrence of a temperature-induced phase transition in mitochondria isolated from apple fruit. *Pl. Physiol., 52,* 390–392.

MCMURDO, A. L. & WILSON, J. M. (1980): Chilling injury and Arrhenius plots. *Cryo-Letter, 1,* 231–238.

MINCHIN, A. & SIMON, E. W. (1973): Chilling injury in cucumber leaves in relation to temperature. *J. exp. Bot., 24,* 1231–1235.

MURATA, T. (1981): Physiological disorders of citrus fruits in Japan. *Proc. Int. Soc. Citriculture, 2,* 776–778.

MURATA, T. (1982): Effect of temperature on the physiological changes of kabosu *(Citrus shaerocarpa* hort. ex Tanaka) fruit during storage. *J. Jap. Soc. hort. Sci., 50,* 516–520.

MURATA, T. (1986): Optimum temperatures for storage of fruits and vegetables with reference to chilling injury. *J. Jap. Ass. Refrig., 3,* 1–11.

MURATA, T. & TATSUMI, Y. (1979): Ion leakage in chilled plant tissues. – in: LYONS, J. M. et al. (Eds) *Low temperature stress in crop plants.* Academic Press, New York, pp. 141–151.

MURATA, T., TATSUMI, Y., IWAMOTO, M. & NISHIMOTO, T. (1980): On the chilling sensitivity of citrus fruits in relation to respiration. *Bull. Fac. Agric. Shizuoka Univ., 30,* 23–27.

NISHIYAMA, I. (1975): A break on the Arrhenius plots of germination activity in rice seeds. *Pl. Cell Physiol., 16,* 533–536.

PURVIS, A. C. (1980): Respiration of grapefruit and orange flavedo tissue in relation to chilling and non-chilling temperatures and respiratory inhibitors. *J. Am. Soc. hort. Sci., 105,* 209–213.

RAISON, J. K. (1973): Temperature-induced phase change in membrane lipids and their influence on metabolic regulation. *Symp. Soc. exp. Biol.* (Cambridge), *27,* 485–512.

RAISON, J. K. & CHAPMAN, E. A. (1976): Membrane phase changes in chilling-sensitive *Vigna radiata* and their significance to growth. *Aust. J. Plant Physiol., 3,* 291–299.

TATSUMI, Y. & MURATA, T. (1981): Relation between chilling sensitivity of *Cucurbitaceae* fruits and membrane permeability. *J. Jap. Soc. hort. Sci., 50,* 108–115.

WILLCOX, M. E. & PATTERSON, B. D. (1979): Breaks or curves ? A visual aid to the interpretation of data. – in: LYONS, J. M. et al. (Eds) *Low temperature stress in crop plants.* Academic Press, New York. pp. 523–525.

YAMAKI, S. & URITANI, I. (1974): Mechanism of chilling injury in sweet potato. – Part XII. Temperature dependency of succinoxidase activity and lipid-protein interaction in mitochondria from healthy or chilling-stored tissue. *Pl. Cell Physiol., 15,* 669–680.

Acta Alimentaria, Vol. 19 (1), pp. 19—26 (1990)

CHARACTERIZATION OF A PECTIN FROM SUNFLOWER HEADS RESIDUES

M. L. Alarcão e Silva

Centro de Microbiologiae Indústrias Agrícólas, Instituto Superior de Agronomia, 1399 Lisboa Codex. Portugal

(Received: 24 January 1989; accepted: 5 June 1989)

The chemical composition of a low methoxyl pectin extracted from sunflower heads residues (yield being 19.24% of original dry matter) was studied and its degree of purity emphasized, considering the very high galacturonic acid content (90.82%). The average molecular weight, $\overline{M}v$, was calculated from intrinsic viscosity (η) measurements on dilute solutions, and was found to be around 105 000. Research on flow behaviour of sunflower pectin solutions was carried out and led to select the power law model ($\tau = k\dot{\gamma}^n$) as the most suitable for fitting the viscometric data at the concentration of 0.5% (w/v). The results presented along with data provided for the commercial pectin used as standard and the specifications of EEC, FAO and National Research Council for this food additive, suggest that sunflower could be an important natural source of low methoxyl pectin with some interesting applications in food technology.

Keywords: flow behaviour, low methoxyl pectin, sunflower heads

Colin and Lemoyne (1940) first reported the presence of high pectin content in the pith of sunflower plant. Since then, numerous authors (Shewfelt & Worthington, 1953; Zitko & Bishop, 1966; Riaz & Uddin, 1972; Lin et al., 1975; Campbell et al., 1978; Pathak & Shukla, 1981) have published their investigations concerning several aspects of sunflower pectin.

The sunflower crop is important for Portuguese agriculture as a source of vegetable oil. So, an evaluation of the potential yield of pectin from sunflower heads residues was made (Alarcão e Silva, 1987). The amounts found justified detailed study of the chemical composition of this pectin in order to find whether there are limitations to its use in food products. With the objective of incorporating the extracted pectin in foods in order to make the best out of its hydrocolloid properties, it seemed necessary to measure some rheological characteristics of sunflower pectin, namely viscometric properties, comparing them with that of a commercial demethylated citrus pectin. Models describing flow behaviour of this pectin solution were studied.

1. Materials and methods

Pectin was obtained from dried heads (without seeds) of sunflower *(Helianthus annuus* L., cv. Arocha) harvested in Vimieiro (Alto Alentejo, Portugal) on three distinct zones of a 10 ha planting area. The extraction of pectin was carried out according to the method of LIN and co-workers (1975). In the present study only the oxalate-soluble pectin was considered since preliminary trials have shown that it was the most interesting for possible industrial use. Pectin was ground to pass through a 60-mesh screen using a Wiley mill, and then purified, following the procedure of the AMERICAN PHARMACEUTICAL ASSOCIATION (1970).

The commecial pectin used as standard was GENU pectin LM-102 AS (partly amidated low methoxyl citrus pectin standardized by blending with sucrose).

1.1. Physico-chemical characterization of pectin

Moisture and ash contents were estimated according to MCCREADY (1970). Acid-insoluble ash was determined by the method of FAO (1978). The value of arsenic in the ash was estimated according to FAO (1978), while lead, copper and zinc were evaluated by atomic absorption spectrophotometry. Nitrogen content was estimated by Kjeldahl semimicro-method (FAO, 1978). Galacturonic acid and methoxyl contents were determined according to the procedures of the AMERICAN PHARMACEUTICAL ASSOCIATION (1970). The degree of esterification was measured as indicated in NATIONAL RESEARCH COUNCIL (1972). Amide content was estimated by micro-Kjeldahl distillation (KIM et al., 1978). Equivalent weight and degree of purity were determined according to the formulae described by DOESBURG (1965). The acetyl content was calculated by the method of OWENS and co-workers (1952). The intrinsic viscosity (η), and viscosity-average molecular weight, $\overline{M}v$, were determined with a Cannon-Fenske capillary viscometer using the procedure of CHRISTENSEN (1954).

1.2. Rheological characterization of pectin (in the form of aqueous solutions)

Flow properties of the pectin solutions, 0.1–0.5% and 0.5% of sunflower and commercial pectins, respectively, were established in a concentric cylinder viscometer (Rheomat 15, Contraves, Zurich) with attached recorder, at a temperature of 20 °C. Flow curves were made by the recorder for both pectins at the concentration of 0.5%, the rotor speeds increasing continuously. For the 0.5% sunflower pectin solution, torque was measured at a complete cycle of rotor speed (5.595 to 352.0 r.p.m.), while for lower concentrations as well

as for the commercial pectin the measurements were made at the speeds of 113.2, 152.0 and 200.0 r.p.m. In order to obtain rheological parameters of non-newtonian fluids based on experimental values, and to select the most suitable model for describing the flow behaviour of the pectin solution, the stepwise procedure reported by COSTELL and DURÁN (1978, 1979), COSTELL (unpublished) was followed.

Using the statistics computer program Andress, developed by Jan A. Andrew (Oxford University) and adapted by R. Sardinha and M. Serafim (Instituto Superior de Agronomia, Lisboa), multiple regression analysis was performed with the apparent viscosity of sunflower pectin solutions as dependent variable and with concentrations and shear rates employed as the independent ones, those variables which did not attain significance being eliminated by a degressive procedure.

2. Results and discussion

2.1. Physico-chemical characterization

The chemical composition and some characteristics of sunflower head pectin are shown in Table 1, along with data provided for commercial pectin (COPENHAGEN PECTIN FACTORY LTD., 1980) and the specifications of EEC (1978, 1982), FAO (1982) and NATIONAL RESEARCH COUNCIL (1972) for this food additive.

The crude pectin content found, 19.24%, was similar to the values reported earlier for other sunflower cultivars (ALARCÃO E SILVA, 1987). This value is higher than those of traditional pectin sources, therefore it appears to be worthy, from an economical point of view, of an eventual industrial extraction. The high galacturonic acid content (90.82%), and the results of other investigations by the author, clearly show that this sunflower pectin is very pure. The results from both the methoxyl content and the degree of esterification showed that it is a low methoxyl pectin (according to the definition of the NATIONAL RESEARCH COUNCIL [NRC], 1972), thus offering advantageous applications in new food products (e.g. low calorie foods). The low ash (1.5%) and acetyl contents (1.95%) are features of a good quality pectin (SABIR et al., 1976; THIBAULT & PETIT, 1979; PILNIK, 1981). The viscosity-average molecular weight was found to be around 105 000, i.e., higher than the value obtained through the same procedure for the commercial demethylated pectin (55 670 for the standardized pectin commercially available and 87 288 for the unstandardized). This characteristic of the sunflower pectin, and the galacturonic acid content mentioned above, may contribute to the high viscosity of pectin solutions and, therefore, to its flow behaviour.

Table 1

Physical and chemical characteristics of sunflower pectin (oxalate-soluble) on a dry purified basis

Composition	Material				Specifications		Nat. Res. Council
	Sunflower pectin			Commercial citric pectin	EEC	FAO	
	$\bar{x}$	n	$\pm$ s				
Yield (% original dry matter)	19.24	10	1.70				
Moisture	6.18	6	0.94	<10	≤12	≤12	≤12
Ash (%)							
Total	1.59	3	0.22	< 7			≤10
HCl insoluble	0.30	3	0.04		≤ 2		≤ 1
Arsenic (ppm)	0.5			< 2		≤ 3	≤ 3
Lead (ppm)	0.4			<10		≤10	≤10
Copper (ppm)	6.4			<25		≤50	
Zinc (ppm)	6.1					≤25	
Total nitrogen (%)	0.33	6	0.01		≤ 2.5	≤ 2.5	
Galacturonic acid (%)	90.82	8	3.87		≥65	≥65	≥35
Methoxyl (%)	3.92	8	0.43				
Esterification (%)	27.03	8	2.80	30			≥50
Amidation (%)	0			20	≤25	≤25	≤40
Equivalent weight	246.63	8	10.51				
Purity (%)	78.49	8	4.06				
Acetyl (%)	1.95	6	0.13				
pH (1% pectin solution)	2.66	3	0.13	3.5—4.5			
Intrinsic viscosity (100 cm^3 g^{-1})	4.93	3	1.07	2.62[a]			
Molecular weight	104 858	3	22 697	55 670[a]			

$\bar{x}$: mean value
n: number of measurements
$\pm$ s: standard deviation
[a] Determined in our laboratory

2.2. *Rheological characterization*

2.2.1. Characterization of flow. Selection of rheological parameters. The flow curve for the sunflower pectin and the standard pectin solutions recorded by the Rheomat 15 viscometer shows distinct behaviours at the same concentration of 0.5% (w/v) (Fig. 1). In the latter, the type of flow was nearly newtonian, whereas the former exhibits shear thinning (pseudoplastic) behaviour (probably with a yield stress).

The values of rheological parameters K (Ns^n m^{-2}) and n for the 0.5% pectin solution using the power law model ($\tau = K\dot{\gamma}^n$) to describe the flow

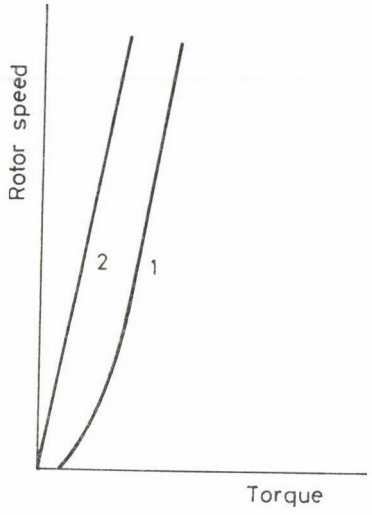


Fig. 1. Flow curves for (1) sunflower pectin and (2) commercial pectin solutions (0.5% w/v), registered by the recorder of viscosimeter Rheomat 15, at a complete cycle of rotor speeds

behaviour and calculated according to the referred methodology were found to be:

$$K = 0.756 \qquad n = 0.49$$

where K is the coefficient of consistency and n is the flow behaviour index.

So the power law equation, with τ (N m^{-2}) and $\dot{\gamma}$ (s^{-1}), assumes the form:

$$\tau = 0.756\dot{\gamma}^{0.49} \qquad (R^2 = 0.998)$$

where τ is the shear stress and $\dot{\gamma}$ is the shear rate. Although that model fitted well, the Hershel-Bulkley model ($\tau = \tau_0 + K\dot{\gamma}^n$) was also tested because it considers the occurrence of a yield stress (τ_0), a value that seemed graphically visible. The parameters of this model were calculated:

$$\tau_0 = 1.102 \qquad K = 0.213 \qquad n = 0.76$$

The equation taking the form:

$$\tau = 1.102 + 0.213\dot{\gamma}^{0.76} \qquad (R^2 = 0.984)$$

Taking into consideration the higher value of R^2 for the power law model and the new investigations indicating that the yield stress concept may be an idealization (BARNES & WALTERS, 1985; LAUNAY et al., 1986) the first model was finally adopted.

2.2.2. Effect of concentration on the apparent viscosity. The influence of pectin concentrations on the apparent viscosities of solutions over the limited

Table 2

Apparent viscosity values of sunflower pectin (S) and commercial pectin (C) at different concentrations and increasing shear rates

Concentration (% w/v)	Shear rate (r. p. m.)					
	113.2		152.0		200.0	
	Viscosity (mPa s)					
	S	C	S	C	S	C
0.1	3.95		4.07		4.25	
0.15	4.99		4.91		4.88	
0.2	8.54		8.30		8.14	
0.4	31.39		28.26		27.07	
0.5	102.49	5.08	89.44	5.04	76.13	5.17

range of shear rates employed in this study (113.2, 152.0, 200.0 r.p.m.) is shown in Table 2.

For sunflower pectin concentrations of 0.2%, 0.15% and 0.1%, solutions showed a remarkable decrease in viscosity values when compared with those of the 0.5% solution. Viscosity remained nearly constant across the reported range of shear rates, i.e., the pectin showed an essentially newtonian behaviour. It is interesting to note that the effect observed on the viscosity of an aqueous solution was nearly the same either upon incorporation of 0.5% of the commercial pectin or 0.15% of the sunflower pectin, and also that levels of sunflower pectin concentrations higher than 0.2% cause a disproportionately higher increase in viscosity values, increases becoming smaller with increased shear rates.

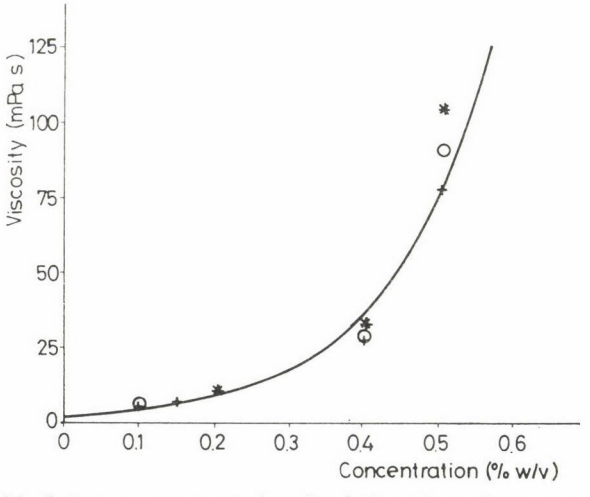


Fig. 2. Relationship between apparent viscosity (η^a) at three shear rates and concentration (c) for the sunflower pectin. $\eta^a = 1.7209e^{7.5736c}$. *: 113.2 r.p.m.; ○: 152.0 r.p.m.; +: 200.0 r.p.m.

Table 3

Analysis of variance of regression equation for apparent viscosity

Source of variation	DF	SS	MS	F
Regression	1	2.033×10	2.033×10	817.81***
Residual	13	3.231×10^{-1}	2.485×10^{-2}	
Total	14	2.065×10		

*** Significant at 0.1% probability level
DF: degree of freedom
SS: sum square
MS: mean square
F: test F

The final model (Fig. 2) calculated for the sunflower pectin according to mathematical statistical method is given:

$$\eta_a = 1.7209e^{7.5736c} \qquad (R^2 = 0.984)$$

where η_a is the apparent viscosity and c the concentration of sunflower pectin.

This model was found to be highly effective for using as a predictive model. Its analysis showed that under the specific conditions of experimentation the tested shear rates lack significance and did not change viscosity behaviours.

Analysis of variance is shown in Table 3.

3. Conclusion

The joint consideration of both chemical and rheological data seems to indicate, as an overall conclusion, that low methoxyl pectin from sunflower heads residues can be an alternative to other commercial demethylated pectins in food technology. However further work is thought advisable in this connection, namely to pursue the investigations on functionality of the new pectin in specially designed low or reduced calorie foods.

*

The author wishes to thank Dr. Luis Durán and Dr. Elvira Costell, Instituto de Agroquímica y Tecnologia de Alimentos (Consejo Superior de Investigaciones Cientificas), Valencia, Spain for the kind permission to carry out some rheological measurements in one of the laboratories.

Literature

Alarcão e Silva, M. L. (1987): *Pectinas em resíduos de girassol. Caracterização e possível aproveitamento em tecnologia alimentar.* Res. Dissertation, Inst. Superior de Agronomia, Lisboa, pp. 53–56; 148–150; 159.

American Pharmaceutical Associaticon (1970): Pectin. — in: *National formulary.* 13th ed. APA, Inc., Washington, D. C., pp. 525–526.

BARNES, H. A. & WALTERS, K. (1985): The yield stress myth? *Rheologica Acta, 24,* 323–326.

CAMPBELL, S. J., SOSULSKI, F. W. & SABIR, M. A. (1978): Development of pectins in sunflower stalks and heads. *Can. J. Pl. Sci., 58,* 863–868.

CHRISTENSEN, P. E. (1954): Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *Fd Res., 19,* 163–172.

COLIN, H. & LEMOYNE, S. (1940): La matiere uronique de la moelle d'*Helianthus. C. r. Acad. Sci., 211,* 44–47.

COPENHAGEN PECTIN FACTORY LTD. (1980): Genu. Product description. P 203–2.

COSTELL, E.: Puesta a punto de métodos sensoriales, instrumentales y químicos para determinar la calidad de conservas, zumos y bebidas. (Unpublished results).

COSTELL, E. & DURÁN, L. (1978): Evaluación y cuantificación de la tixotropia del puré de albaricoque. *Rev. Agroquim. & Tecnol. Aliment., 18,* 470–484.

COSTELL, E. & DURÁN, L. (1979): Reologia del puré de albaricoque. Selección del método de medida del umbral de fluencia. *Rev. Agroquim. & Tecnol. Aliment., 19,* 89–101.

DOESBURG, J. J. (1965): *Pectic substances in fresh and preserved fruits and vegetables.* I. B. V. T. Communication No. 25. Inst. for Research on Storage and Processing of Horticultural Produce. Wageningen, The Nethherlands, pp. 11; 55.

EEC/CEE (1978): Directive 78/663/CEE. J. O. No. L 223, pp. 16–17.

EEC/CEE (1982): Directive 82/504/CEE. J. O. No. L 230., p. 35.

FAO (1978): *Guide to specifications for general notices, general methods identification tests, test solutions and other reference materials.* (FAO food and nutrition paper 5). Food and Agriculture Organization, Rome, pp. 22–24; 33.

FAO (1982): *Normes d'identité et de pureté: pour des solvants entraineurs, des émulsifiants et stabilisants, des preparations d'enzymes, des aromatisants, des colorants alimentaires, des édulcorants et d'autres additifs alimentaires.* (Étude FAO: Alimentation et Nutrition 19). Food and Agriculture Organization, Rome, pp. 11–13.

KIM, W. J., SMIT, C. J. B. & RAO, V. N. M. (1978): Demethylation of pectin using acid and ammonia. *J. Fd Sci., 43,* 74–78.

LAUNAY, B., DOUBLIER, J. L. & CUVELIER, G. (1986): Flow properties of aqueous solutions and dispersions of polysaccharides. – in: MITCHELL, J. R. M. & LEDWARD, D. A. (Eds) *Functional properties of food macromolecules.* Elsevier Applied Science Publishers, London, pp. 1–78.

LIN, M. J. Y., SOSULSKI, F. W., HUMBERT, E. S. & DOWNEY, R. K. (1975): Distribution and composition of pectins in sunflower plants. *Can. J. Pl. Sci., 55,* 507–513.

MCCREADY, R. M. (1970): Pectin. – in: JOSLYN, M. A. (Ed.) *Methods in food analysis* 2nd ed., Academic Press, New York, p. 578.

NATIONAL RESEARCH COUNCIL (1972): Pectin. – in: *Food chemicals codex.* 2nd ed. National Academy of Sciences, Washington, D. C., pp. 577–581.

OWENS, H. S., MCCREADY, R. M., SHEPHERD, A. D., SCHULZ, T. H., PIPPEN, E. L., SWENSON, H. A., MIERS, J. C., ERLANDSEN, R. F. & MACLAY, W. D. (1952): *Methods used at Western Regional Laboratory for extraction and analysis of pectic materials.* Report No. 340, United States Department of Agriculture. Bur. Agric. Ind. Chem., Albany, California, pp. 1–24.

PATHAK, D. K. & SHUKLA, S. D. (1981): Quantity and quality of pectin in sunflower at various stages of maturity. *J. Fd Sci. Technol., 18,* 116–117.

PILNIK, W. (1981): Pectic substances. – in: *Fiber in human nutrition. European Symposium on Fiber in Human Nutrition.* Paris, APRIA, Paris, pp. 91–125.

RIAZ, S. & UDDIN, M. (1972): Polysaccharide components of sunflower heads. – Part 1. The pectins. *Pakist. J. scient. ind. Res., 15,* 167–170.

SABIR, M. A., SOSULSKI, F. W. & CAMPBELL, S. J. (1976): Polymetaphosphate and oxalate extraction of sunflower pectins. *J. agric. Fd Chem., 24,* 348–350.

SHEWFELT, A. L. & WORTHINGTON, O. J. (1953): The extraction of pectin from sunflower heads. *Fd Technol., 7,* 336–340.

THIBAULT, J. F. & PETIT, R. (1979): Les substances pectiques: généralités et domaine d'application dans les industries alimentaires. *Ind. aliment. agric., 96,* 1231–1240.

ZITKO, V. & BISHOP, C. T. (1966): Structure of a galacturonan from sunflower pectic acid. *Can. J. Chem., 44,* 1275–1282.

Acta Alimentaria, Vol. 19 (1), pp. 27—40 (1990)

MINERAL ELEMENT CONTENT OF EDIBLE AND POISONOUS MACROFUNGI

J. VETTER

Department of Botany, University of Veterinary Science,
H-1400 Budapest, Pf. 2. Hungary

(Received: 25 January 1989; accepted: 9 May 1989)

The mineral composition of the fruiting body of macrofungi was studied in 40 samples of 29 edible species and 10 samples of 8 poisoning species. All the samples were tested for 20 elements. Some characteristics and, from the point of view of utilization, useful properties of the fungi and relationships are summarized in the following:

— A significant bioaccumulation of arsenic was observed in the Agaricus species, in *Coprinus atramentarius*, *Langermannia gigantea*, *Lepista nuda*, *Macrolepiota rhacodes* (26.6 ppm) species. Luckily *Agaricus bisporus* (cultivated mushroom) was not found to accumulate arsenic.

— The toxicologically important accumulation of cadmium was proven for a group of the Agaricus species, for a Russula species and also for *Tricholoma terreum*.

— While some genera are characterized by the accumulation of copper (Macrolepiota, Agaricus) others suffer from substantial lack of copper (Gomphidius). Wood destroying fungi species and genera generally show a low copper level.

— An important character of all the macrofungi studied was a well-balanced potassium content in relation to other elements. A high potassium concentration in the family Amanitaceae seems to be a proven chemotaxonomic characteristic.

— One characteristic of all the species investigated (edible as well as poisoning) which distinguishes them from the chemical composition of green plants is the relatively low manganese content (proportion of copper to manganese).

— The Boletus and Marcolepiota species were found to accumulate selenium.

— The only species found to accumulate vanadium was *Amanita muscaria*.

The mineral content of the respective groups of edible and poisoning macrofungi did not differ as established on the basis of the species representing the two groups. The differences observed reside mainly in their absorbing, retaining and storing capacity which, on the other hand, is a function of their taxonomical place, that is a property of chemotaxonomic character. Data and correlations here described may serve with useful guidance in the practice (collection, cultivation or canning of edible fungi) with special reference to some elements present of toxicological importance or decisive of product quality.

Keywords: mineral elements, bioaccumulation, edible fungi, poisonous fungi

Of the foodstuffs of animal and plant origin, perhaps macrofungi were the latest to be thoroughly examined for their composition. First they were analyzed in view of their chemical composition: their moisture, protein and carbohydrate content and fat content were determined and various more easily measurable mineral elements (e.g. alkali earth metals). Related literature utilized some publications or data taken from monographs. Later more modern

methods were applied to determine the quantity of individual elements in certain fungi (strontium: SEEGER et al., 1982; tallium: SEEGER & GROSS, 1981; cadmium: SEEGER, 1978; selenium: QUINCHE, 1983; PIEPPONEN et al., 1983; vanadium and molybdenum: MEISCH et al., 1978; radioactive elements: HASELWANDTER, 1977; BENDE & SZABÓ, 1974). Other investigations were carried out to establish the mineral composition of macrofungi characteristic of a certain area or available in large quantities (HINNERI, 1975, south-western Finland; SOLOMKO et al., 1986, Ukraine; TYLER, 1980, Sweden; SANTOPRETE & INNOCENTI, 1984, surroundings of Bologna).

At the end 1970s and the beginning of 80s emerged first complex problems in relation to the mineral composition of macrofungi, such as the existence of relationships between their place in taxonomy, type of nutrition, age on the one hand and their mineral composition on the other. Complex investigations were extended over several elements (TYLER, 1980; 1982) and many species (samples). In recent years researchers interested in food analysis and nutrition biology invested special interest in certain macrofungi said to have the capacity to accumulate high amounts of certain elements (unfortunately often of toxic effect). Thus, particular attention was paid to cadmium (ENKE et al., 1977; SCHMITT & MEISCH, 1985; LAUB et al., 1977), mercury (LAAKSOVIRTA & LODEUIUS, 1979; LAAKSOVIRTA & ALAKUIJALA, 1978) and lead (SEEGER et al., 1976).

Research related to this subject is still not extensive in Hungary. VASS and TÖLGYESI (1979) and TÖLGYESI and VASS (1984) have analyzed the mineral element composition of certain plant and fungal species in order to compare the results VETTER and KONECSNI (1981) carried out the analyses of nearly 100 commercial dried mushroom samples, determining the amount of 7 mineral elements in them. GERGELY and co-workers (1986) determined the microelement content of some edible mushrooms. The authors carried out a series of analyses during 1987–88, studying 20 mineral elements in macrofungi samples coming from different parts of the country. The investigation included not only the analysis of the macrofungi species but problems of correlations between their mineral element content and their type of nutrition or the substrate (wood, soil) and the mineral content of the fungus, etc.

The present study utilizing the available data base, presents the mineral element composition of the most important Hungarian edible mushrooms and some important poisoning macrofungi (using macrofungus samples from different parts of the country). Our aim was, among others, to draw attention to the fact or possibility of accumulation of some nutrition-biologically or toxicologically important elements.

1. Materials and methods

The samples were collected in different regions of the country in the years 1984 to 1987. The samples represent the most important and generally frequently occurring mushroom species. Three Agaricus species (*A. augustus* Fr., *A. arvensis* Schff. ex Fr., *A. xanthoderma* Gen and cultivated *Agaricus bisporus* Lge. (Singer), *Armillariella mellea* (Vahl. in Fl. Dan. ex Fr.) Karst., *Boletus edulis* Bull. ex Fr., *Cantharellus cibarius* Fr., *Clitocybe nebularis* (Fr.) Harmaja, *Clitocybe odora* (Bull. ex Fr.) Kummer, *Coprinus atramentarius* (Bull. ex Fr.) Fr., *C. micaceus* (Bull. ex Fr.) Fr., *Flammulina velutipes* (Curt. ex Fr.) Sing., *Gomphidius glutinosus* (Schff.) Fr. *Hirneola auricula-judae* (Bull. ex St.-Anm.) Wettst., *Laetiporus sulphureus* (Bull. ex Fr.) Murill, *Lactarius piperarus* (L. ex Fr.), *Langermannia gigantea* Batsch. Pers. (Rostk), *Lepista nuda* (Bull. ex Fr.) Che, *Macrolepiota procera* (Scop. ex Fr.) Sing, *M. rhacodes* (Vitt.) Sing., *Pleurotus ostreatus* (Jacq. ex Fr.), *P. pulmonarius* Fr., *Russula cyanoxantha* Schff. ex Fr., R. vesca Fr. *R. xerampelina* (Sch. ex Secr.) Fr., *Suillus granulatus* (L. ex Fr.) Kummer, *Tricholoma terreum* (Schff. ex Fr.) Kummer, *Xerocomus chrysentheron* (Bull. ex St. Amans) Quél. and *Hypholoma capnoides* (Fr.) ex Fr. Kummer, were analyzed.

Poisonous fungi were represented by the following: *Amanita phalloides* (Vaill. ex Fr.), *A. verna* (Bull. ex Fr.) Pers. ex Vitt., *A. muscaria* (L. ex Fr.) Hooker, *A. rubescens* (Pers. ex Fr.) Gray, *A. vaginata* (Bull. ex Fr.) Gray, *Boletus luridus* (Schff. ex Fr.), *Entoloma sinuatum* Kummer and *Hypholoma sublateritium* (Fr.) Quél.

The fruiting bodies were collected by lifting them out of the soil or in case of wood destroying fungi, cutting them off their substratum. Contamination with soil was removed either by cutting of the soiled part of the stem or by mechanical cleaning of the fruiting body or by washing it in tap-water, distilled water or twice distilled water. Longer soaking, however, was not applied because the possibility of dissolving and thereby removing some of the more mobile mineral elements. After cleaning the fruiting bodies were sliced, dried under mild conditions and milled to flour fineness. The samples were then macerated with HNO_3–H_2O_2 in special teflon vassels under 1.56×10^5 P pressure for 30 min (200 mg pulverized ssmple, 2 cm^3 HNO_3, 2 cm^3 H_2O_2). The macerated substance was filtered and diluted to 10 cm^3, with twice distilled water. The quantity of the mineral elements was determined by plasma-generated spectroscopy, using an ICAP type 9000 apparatus at the Chemistry Department, University of Horticulture and Food Industry, Budapest.

Determinations were carried out in three parallels. To achieve better overview of the data as well as because the standard deviation between measurements (except for a few elements present in minute quantities) did not exceed a few percentages, only the arithmetic means were listed in the Tables.

2. Results and discussion

Data are given separately for edible mushrooms and the toxic fungi. Results relate to the absolute dry weight of the samples in ppm (mg per kg). Data related to *Agaricus xanthoderma* species were put on the list of edible mushrooms along the other Agaricus species in order to demonstrate the taxonomic correlations. *Amanita rubescens* was put for the same reason among the toxic Amanita species.

A survey of the tabulated results shows some important conclusions as regards the elements constituting the macrofungi.

The aluminium content of the fungi varied within a wide range and no correlation was found between it and the type of nutrition, the place in taxonomy or the habitat of the fungi. The differences in the arsenic content of the fungi, however, show important relationships. In the majority of the samples the amount of arsenic did not even reach the lowest limit of detection, but in some species a significant amount was found. The three Agaricus species contained arsenic (while none was found in the cultivated *A. bisporus*) and *Coprinus atramentarius*, *Langermannia gigantea*, *Lepista nuda* contained some arsenic and *Macrolepiota rhacodes* the highest amount, 26.6 ppm. The observation of the authors on the arsenic content of a few ppm in *Langermannia gigantea* corroborates the findings of RIMÓCZI (1987). The accumulation of arsenic in the other species has not been mentioned in related literature so far. The suggestion that the accumulation of arsenic is in connection with the habitat is most unlikely since other species collected in the same region do not contain arsenic. It is interesting to note that no arsenic could be detected in any of the toxic species.

The boron content varied also within wide limits (0–62 ppm). High amounts were detected in *Lepista nuda*, *Clitocybe odora* species and little in some of the fungi living on wood (*Flammulina velutipes*, *Hirneola auricula-judae*, *Laetiporus sulphureus*). A direct relationship between the boron content found and the place of the species in taxonomy could not be detected.

The barium content of fungi varied between low limits. Of the edible mushrooms a sample of *Clitocybe nebularis* contained 12 ppm, *Coprinus micaceus* 15.9 ppm and *Hirneola auricula-judae* 19.2 ppm barium. The lowest values were found in *Lactarius piperatus* (1.96 ppm) and *Tricholoma terreum* (1.44 ppm). Most of the samples had low amounts of barium.

The calcium content of the samples was varied, too. The highest level was measured in two Coprinus species and in *Hirneola auricula-judae* (4–9000 ppm). High values were measured in cultivated *A. bisporus* D-13 strain, in the stipe and pileus (2800 and 2300 ppm, respectively). It seems that species living on trees (Pleurotus, Flammulina) have a higher Ca content than those destroying dead leaves or the mycorrhiza species. The lowest level

Table 1

Composition of mineral elements in edible mushrooms
(ppm in dry matter)

Name of fungus	Habitat	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K
Agaricus xanthoderma	Mountain Bükk	191.5	3.5	0.88	6.09	1537	0.60	0	0	147.1	301	43119
A. augustus Fr.	Csillebérc	54.5	11.9	0.93	3.14	329	15.00	3.60	0	61.3	107	27401
A. arvensis	Mountain Bükk	159.7	8.3	4.12	3.56	1142	17.30	0.82	1.27	153.4	258	52126
A. bisporus	cultivated "D-13"											
pileus		70.2	0	25.20	2.32	2829	0.18	0	1.20	61.7	78	41132
stipe		40.3	0	17.85	2.39	2371	0.24	0.09	1.00	41.6	76	35534
Armillariella mellea	Mountain Mátra	57.2	0	0.53	2.37	774	2.53	0	0.85	10.8	182	51180
	Hanság	73.1	0	3.58	2.38	1088	0.91	0	0.67	13.6	135	42158
Boletus edulis	Csillebérc	60.1	0	4.12	2.79	822	5.22	0	0.78	18.6	109	24818
	Budakeszi	26.4	0	5.68	2.23	791	1.02	0	1.21	15.9	63	21743
Cantharellus cibarius	Mountain Börzsöny	116.1	0	17.30	3.61	536	0.28	0	0	36.8	113	41045
Clitocybe nebularis	Mountain Bükk/1	52.7	0	7.47	2.42	833	2.28	0	1.18	78.2	186	25959
	Bükk/2	297.0	0	1.00	12.06	2049	0.36	0.36	2.75	24.1	428	36437
C. odora	Pilisszentkereszt	111.9	0	47.00	6.48	1415	1.04	0.13	1.34	165.3	172	30370
Coprinus atramentarius	Mountain Zemplén	119.1	11.8	0	8.39	4235	0.61	0.28	0.87	31.2	176	55749
C. micaceus	Normafa	690.4	0	0.61	15.90	9147	0.77	0.19	0.82	31.7	995	68883
Flammulina velutipes	Érd/1	309.0	0	6.49	4.72	3453	2.99	0.25	1.44	6.6	435	39725
	Érd/2	58.2	0	0.74	2.77	1459	1.26	0.04	1.09	7.3	79	30481
Gomphidius glutinosus	Pilis	67.0	0	0.93	1.90	244	0.20	0	1.23	3.7	127	37604
Hirneola auricula-judae	Normafa	235.5	0	2.23	19.20	7520	0.59	0.18	1.22	7.2	331	4321
Laetiporus sulphureus	Mountain Pilis	50.6	0	0	4.38	726	0.38	0.19	0.60	6.2	63	27405
Lactarius piperatus	Királyrét	43.7	0	1.4	1.96	274	3.32	0	0	45.9	61	24556
Langemannia gigantea	Mountain Bükk	412.0	7.1	0	3.69	1462	1.64	0.69	1.32	33.5	461	24679

Table 2

Composition of mineral elements in edible mushrooms (Continuation)
(ppm in dry matter)

Name of fungus	Habitat	Mg	Mn	Ni	P	Se	Sr	Ti	V	Zn
Agaricus xanthoderma	Mountain Bükk	1299	25.6	1.66	11258	2.26	3.57	3.00	0	103.6
A. augustus Fr.	Csillebérc	906	9.1	4.33	7719	0	1.09	1.25	0	70.6
A. arvensis	Mountain Bükk	1672	25.9	4.60	13956	4.60	7.19	2.24	0.32	130.5
A. bisporus	cultivated "D-13"									
pileus		1236	8.3	1.42	14311	0	9.82	0.35	0	93.4
stipe		907	6.3	1.35	9694	0	9.37	0.22	0.08	81.4
Armillariella mellea	Mountain Mátra	1432	22.3	0.87	8004	0	6.54	0.75	0	69.7
	Hanság	1179	11.9	1.08	5618	0	6.99	1.53	0	93.4
Boletus edulis	Csillebérc	823	13.2	4.11	6374	30.20	5.99	1.38	0	152.0
	Budakeszi	668	14.6	3.62	4501	11.20	5.97	0.54	0.55	85.9
Cantharellus cibarius	Mountain Börzsöny	1019	20.3	0.72	4587	0	2.17	2.54	0.23	59.8
Clitocybe nebularis	Mountain Bükk/1	1122	17.9	1.84	10903	0	4.62	1.01	0.40	77.7
	Bükk/2	1446	118.6	2.84	5043	0	9.66	7.72	1.08	98.7
C. odora	Pilisszentkereszt	1330	55.3	2.74	13805	0	8.77	1.28	1.51	103.9
Coprinus atramentarius	Mountain Zemplén	1876	13.6	6.72	9674	0	21.12	0.92	0.07	60.9
C. micaceus	Normafa	5111	44.6	2.29	8368	0	12.53	11.97	1.14	69.3
Flammulina velutipes	Érd/1	3074	21.7	2.47	8953	0	15.52	9.28	2.51	91.7
	Érd/2	1642	12.6	2.12	10011	0	10.65	0.89	1.20	125.1
Gomphidius glutinosus	Mountain Pilis	1055	22.9	2.26	6603	0	0.67	1.74	0	39.1
Hirneola auricula-judae	Normafa	2547	27.7	1.94	1939	0	33.60	6.71	1.71	56.7
Laetiporus sulphureus	Mountain Pilis	1012	4.4	0.86	4484	0.76	6.48	0.45	0.10	52.2
Lactarius piperatus	Királyrét	669	12.8	0.64	4376	0	1.16	1.28	0	66.1
Langermannia gigantea	Mountain Bükk	1446	27.1	4.55	13683	0	6.32	7.11	0.67	192.1

Table 3

Mineral elements in edible mushrooms (Continuation)
(ppm in dry matter)

Name of fungus	Habitat	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K
Lepista nuda	Őriszentpéter	253.6	5.4	62.9	7.94	570	0.51		1.33	84.1	133	38296
Macrolepiota procera	Salgótarján Mountain Karancs											
	young pileus	36.6	0	1.85	1.95	919	2.02		0.67	226.8	103	30152
	young stipe	46.1	0.	8.53	4.95	187	0.56		0	121.4	105	33441
	aged pileus	42.6	0	10.60	4.01	738	1.03		0	116.5	119	27082
	aged stipe	37.7	0	1.95	4.33	700	1.26		0	170.4	86	25043
M. rhacodes	Mountain Bükk	86.7	26.6	31.40	3.69	1020	1.14	0.48	1.02	116.5	216	34838
Pleurotus ostreatus	Herend	24.4	0	5.61	16.00	1596	2.18	0	0	6.9	99	23296
	Érd	128.5	0	5.85	4.40	1651	1.16	0.07	1.18	11.0	252	27117
	Gemenc	58.1	0	4.30	2.60	1401	1.37	0	0.62	5.7	115	19930
P. pulmonarius	Mountain Bükk	49.7	0	0	2.80	808	1.89	0.19	1.05	21.7	158	40205
Russula cyanoxantha	Budakeszi	118.4	0	27.6	2.74	335	8.71	0.46	0.76	45.7	191	31765
R. vesca	Pilisszentkereszt	142.4	0	3.6	5.38	783	12.40	0.50	0.70	47.7	230	27323
R. xerampelina	Pilisszentkereszt	137.8	0	2.87	5.52	663	5.25	0	0.68	65.4	252	30777
Suillus granulatus	Pilisszentkereszt	319.8	0	6.8	4.39	397	0.69	0	0	17.4	263	31311
Tricholoma terreum	Pilisszentkereszt	96.2	0	5.8	1.44	332	13.84	0	1.06	35.4	144	46656
Xerocomus chrysenteron	Pilisszentkereszt	128.5	0	32.8	4.58	1051	0.19	0.20	0.93	46.2	324	47206
Hypholoma capnoides	Mountain Pilis	81.9	0	0	6.16	1258	1.00	0.2	7.05	58.1	193	38011

Table 4

Mineral elements in edible mushrooms (Continuation)
(ppm in dry matter)

Name of fungus	Habitat	Mg	Mn	Ni	P	Se	Sr	Ti	V	Zn
Lepista nuda	Őriszentpéter	1596	98.2	1.39	19382		1.91	1.29		111.3
Macrolepiota procera	Salgótarján Mountain Karancs									
young pileus		1256	13.3	0.94	11539	5.83	6.10	0.73	0	95.6
young stipe		1006	37.2	1.38	7362	0	2.28	0.99	0	62.8
aged pileus		1205	19.9	0.98	9721	0	2.04	0.84	0	93.7
aged stipe		1051	11.7	1.60	8386	0	2.02	0.67	0	72.9
M. rhacodes	Mountain Bükk	1471	40.8	2.91	13172	7.61	7.30	1.49	0.42	142.7
Pleurotus ostreatus	Herend	1429	23.6	1.88	7132	0	5.98	0.54	0	83.9
	Érd	1590	12.5	1.85	6319	0	10.25	3.14	0.74	91.2
	Gemenc	1204	9.7	1.30	3792	1.05	8.13	0.99	0.12	70.3
P. pulmonarius	Mountain Bükk	1965	31.6	1.45	12846	0	6.57	0.52	0	115.5
Russula cyanoxantha	Budakeszi	682	31.9	1.61	3871	0	0.98	2.16	0.28	61.2
R. vesca	Pilisszentkereszt	739	40.6	2.20	4890	0	2.25	3.53	0.27	106.4
R. xerampelina	Pilisszentkereszt	876	35.4	1.43	5138	0	1.81	3.19	0.29	91.1
Suillus granulatus	Pilisszentkereszt	992	16.7	1.10	4965	0	1.64	5.02	0.29	107.0
Tricholoma terreum	Pilisszentkereszt	1313	6.7	1.41	5822	0	0.80	0		121.7
Xerocomus chrysentheron	Pilisszentkereszt	1154	30.2	4.53	10123	0	6.94	2.99	0.28	125.2
Hypholoma capnoides (Fr.) ex Fr.	Mountain Pilis	1267	58.9	1.96	8451	0	9.55	1.43	0.15	88.4

was measured in *Lactarius piperatus* (274 ppm) and *Gomphidius glutinosus* (244 ppm).

The cadmium level in Hungarian macrofungi and important from the aspect of toxicology was found rather heterogeneous, which is in harmony with data published in related literature. Cadmium accumulating capacity is characteristic of only some of the taxonomical categories. While most of the fungi contain only a few tenth or 1–2 ppm Cd, *Agaricus augustus* and *A. arvensis* species have 15–17 ppm. Data published elsewhere show that *A. purpurellus* and *A. silvaticus* have a Cd content of 86 ppm. *Amanita muscaria* contains 22 ppm, *Russula vesca* 12.4 ppm, *Tricholoma terreum* 13.8 ppm cadmidum. According to VETTER (1987) and the data established in this study certain taxonomic categories (primarily the genus Agaricus) have a definite Cd binding capacity which is in connection with the presence of Cd-mycophosphatine, a Cd binding protein. At the same time it is interesting to note the observation — although requiring further confirmation — that this unfavourable property is valid within the genus only for the Flavescentes group (LAUB et al., 1977). The fact that of the samples studied *A. xanthoderma* and *A. bisporus* have a negligably low Cd level seems to prove the above assumption. At present there is no explanation available for the fact that while *Russula vesca* contained 12.4 ppm cadmium the other 12 Russula species investigated did not show Cd accumulating capacity (VETTER, 1987). In the case of *Amanita muscaria* (see later) the accumulation of cadmium is in connection with the accumulation of other elements (V, Cu).

Low values were measured generally related to cobalt (some tenth of ppm). *A. augustus* with its 3.6 ppm Co content is high above the average. Generally little chromium was found in the samples tested, however it varied between 0 and 2.75 (the latter was found in a *Clitocybe nebularis* sample) but no systematic correlation could be observed.

The copper content of the samples varied within a very wide range (*Gomphidius glutinosus* contained 3.7 ppm Cu while *Macrolepiota procera* 226 ppm). Unambiguously high copper accumulation was measured in all the *Macrolepiota procera* and *M. rhacodes* samples, in the Agaricus species studied samples were found containing 260 ppm copper and the 165 ppm found in *Clitocybe odora* is also high. In accordance with the data of TYLER (1980) *Gomphidius glutinosus* was found characteristically lacking in copper as all the wood destroying fungi (Hirneola, Laetiporus, Flammulina, Armillariella, Pleurotus and Hypholoma genera).

Although the values of iron content fluctuate substantially, correlation of taxonomic character could not be established. It seems, however, that the species living on trees have an even lower Fe content in comparison to other species belonging to different types of nutrition.

The element present in higher fungi in highest concentration is potassium.

It is interesting to note that data found in the literature and based on a large number of samples are very close. TYLER (1980) analyzed 130 samples and the average amount of potassium was 32 000 ppm, TÖLGYESI and VASS (1984) found 31 700 ppm as the average of 39 samples. The average value found by VETTER and KONECSNI (1981) based on the analysis of 80 samples was 31 800 ppm. In a more recent analysis of 80 samples VETTER (1987) found an average value of 33 456 ppm. The potassium content independently of place in taxonomy, habitat and other differences seems to be the most balanced mineral component in comparison to the majority of other elements. The high potassium content of the family of Amanitaceae was considered by TÖLGYESI and VASS (1984) an important chemotaxonomical mark and this was confirmed by the present study, too. All species belonging to Amanitaceae, except *A. muscaria* had a very similar potassium content independently of their place of origin. The highest value was measured in *Entoloma sinuatum* (88 474 ppm) and the lowest value belonged to *Hirneola auricula-judae* (4321 ppm). It seems a general correlation that wood destroying fungi have a lower K content (Tables 1–4).

Magnesium belonging to group II of the mineral elements was found to be in the majority of samples between 1000 and 1500 ppm. In a few of the samples it was below this level: in Russula species 680–870 ppm, in *Boletus edulis* 660–823 ppm. The highest amount was measured in *Hirneola auricula-judae, Flammulina velutipes* and *Coprinus micaceus* (5111 ppm). These observations corroborate those of TYLER (1980) carried out in Sweden. He found relatively low values in Russula. Boletus and Lactarius species and more or less higher values in the Agaricus and Coprinus species.

TÖLGYESI and VASS (1984) and earlier researchers considered an important characteristic of fungi distinguishing them sharply from green plants their low manganese content. The analyses of 80 samples (VETTER, 1987) gave an average value of 28 ppm and a more recent analysis of commercial dried mushrooms (VETTER & KONECSNI, 1981) a value of 40 ppm, reflecting an observation similar to that of TÖLGYESI and VASS (1984). While the manganese content of the majority of the samples was around 30, at most 40 ppm, some strikingly low values were measured, for instance 4.4 ppm in *Laetiporus sulphureus* or 6.3–8.3 ppm in *Agaricus bisporus* and a very high value of 118 ppm in *Clitocybe nebularis*.

The average nickel content of Hungarian fungi proved to be higher (2.0 ppm) than that of Italian fungi (SANTOPRETE & INNOCENTI, 1984) or of Swedish fungi (TYLER, 1980) or of Ukrainian fungi (SOLOMKO et al., 1986). The highest values were measured in *Boletus luridus* and in *Xerocomus chrysentheron*, which is not too distant taxonomically.

The second highest mineral element in fungi is phosphorus. Its quantity seems to be relatively well balanced, exceeding 10 000 ppm only in a few

Table 5

Composition of mineral elements in poisoning fungi
(ppm in dry matter)

Name of fungus	Habitat	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K
Amanita phalloides	Karancs	181.2	0	16.3	3.88	1031	0	0	0	33.6	289	47226
	Őriszentpéter	283.0	0	26.0	3.21	597	0.37	0	0.58	38.8	158	45418
	Pilisszentkereszt	149.0	0	3.5	5.31	892	1.10	0	0.73	39.9	339	46199
Amanita verna	Halmi erdő	343.9	0	0.6	5.51	944	0.42	0	0	32.7	489	47639
A. muscaria	Mountain Bükk	76.1	0	21.6	2.91	1065	22.2	1.4	0	25.6	130	27220
A. vaginata	Csillebérc	139.3	0	8.0	6.07	804	4.54	0	0	39.1	328	45420
A. rubescens	Csillebérc	260.0	0	5.5	6.44	1434	0.99	0	1.46	39.7	305	58301
Boletus luridus	Csillebérc	50.9	0	8.2	2.12	691	0.60	0	0.75	30.2	88	20890
Entoloma sinuatum	Mountain Börzsöny	552.9	0	0	5.06	739	3.89	0.7	0	17.3	644	88474
Hypholoma sublateritium	Szarvaskút	38.5	0	0	2.00	803	0.63	0.1	0.82	12.2	81	26178

Table 6

Composition of mineral elements in poisoning fungi (Continuation)
(ppm in dry matter)

Name of fungus	Habitat	Mg	Mn	Ni	P	Se	Sr	Ti	V	Zn
Amanita phalloides	Karancs	866.5	46.6	0	5241	0	6.59	0	0	36.3
	Őriszentpéter	957.7	25.7	0.68	6510	0	1.95	0.80	0.57	39.8
	Pilisszentkereszt	907.0	40.0	1.23	5474	0	2.33	3.38	0.36	36.7
Amanita verna	Halmi erdő	979.1	41.1	0.69	5481	0	3.07	8.24	0.19	41.9
A. muscaria	Mountain Bükk	744.3	14.8	2.40	7145	0	6.99	2.28	56.8	121.8
A. vaginata	Csillebérc	1174.0	14.4	1.01	4982	0	2.50	3.39	0.36	115.5
A. rubescens	Csillebérc	1256.5	50.9	3.30	8433	0	7.75	4.30	0.55	147.8
Boletus luridus	Csillebérc	1029.1	6.4	2.75	6271	6.9	5.13	0.88	0	132.9
Entoloma sinuatum	Mountain Börzsöny	2032.0	52.2	1.03	10534	0	2.79	12.92	0.33	125.6
H. sublateritium	Szarvaskút	969.1	6.3	1.45	5079	0	6.89	0.64	0.06	63.8

samples. The lowest values were found in the wood destroying fungi, similarly to other mineral elements, e.g. in *Hirneola auricula-judae* 1939 ppm and in the genus Russula. The highest values were found in samples of the Agaricus species, and the highest of all *Lepista nuda* (19 382 ppm).

Some authors described a high selenium accumulation in certain fungal species (SOLOMKO et al., 1986; QUINCHE, 1983), particularly in Boletus species. In this study selenium could not even be detected in the majority of the samples (38 samples), however in some samples high values were measured. In two *Boletus edulis* samples 11 and 30 ppm, respectively, were found, in *Boletus luridus* 6.9 ppm, in *Macrolepiota procera* and *M. rhacodes* 5.8–7.6 ppm. These findings support the assumption that the genus Boletus accumulates selenium and this fact, taking into account that our food of plant origin is poor in selenium, increases the value of these species which are otherwise too appreciated.

The strontium content of the samples was substantially lower than that of green plants (VETTER & HARASZTI, 1981). Some of the samples, mostly originating from places near Budapest accumulated strontium. The highest value was found in *Hirneola auricula-judae* (33.6 ppm) and the Flammulina and Coprinus species.

Significant differences were observed in the vanadium content of fungi. The majority of the samples contained none or a few tenth and a higher value than 1 ppm was obtained only in 5 samples and really outstanding value in *Amanita muscaria* (56 ppm). Thus, vanadium accumulation is characteristic only of a single species of a genus in which the amavadin named acceptor compound is capable of binding a substantial amount of metal. More recent samples collected in other regions (mountain Börzsöny) of the country gave a much higher vanadium content 176 ppm (!) in the pileus and 143 ppm in the stipe (Tables 5–6).

The zinc content of fungi shows a rather well balanced picture in comparison to other elements (the average of edible mushrooms was 90 ppm and that of toxic fungi 86 ppm), neither bioaccumulation nor lack of zinc was observed in the samples analyzed. Somewhat lower values were found in fungi living on trees and in certain Amanita species. In earlier analyses slightly higher values were measured in some Agaricus species. The maximum amount of zinc was measured in *Russula atropurpurea* (657 ppm), however, no explanation was found for this yet.

*

The author wishes to express here his acknowledgement for the help rendered by Dr. P. FODOR, Chemistry Department, University of Horticulture and Food Industry, Budapest.

Literature

BENDE, E. & SZABÓ, A. (1974): Egyes gombák radioaktív szennyezettsége. (Radioactive contamination in certain fungi.) *Mikol. Közl., 12,* 91–94.

ENKE, M., MATSCHINER, H. & ACHTZEHN, M. K. (1977): Schwermetallanreichungen in Pilzen. *Nahrung, 21,* 331–334.

GERGELY, A., MASAS, G., MILOTAI, G. & LEBOVICS, V. (1986): Néhány ehető gomba mikroelemtartalma. (Microelement content of some edible mushrooms.) *Mikol. Közl. (Clusiana), 24,* 125–131.

HASELWANDTER, K. (1977): Radioactives (^{137}Cs) in Fruchtkörpern verschiedener Basidiomycetes. *Z. Pilz., 43,* 323–326.

HINNERI, S. (1975): Mineral elements of macrofungi in oak-rich forests of Lenholm island, inner archipelago of SW Finland. *Ann. Bot. Fenn., 12,* 135–140.

LAAKSOVIRTA, K. & ALAKUIJALA, P. (1978): Lead, cadmium and zinc contents of fungi in the parks of Helsinki. *Ann. Bot. Fenn., 15,* 253–257.

LAAKSOVIRTA, K. & LODENIUS, M. (1979): Mercury content of fungi in Helsinki. *Ann. Bot. Fenn., 16,* 208–212.

LAUB, E., WALIGORSKI, F., WOLLER, R. & LICHTENTHAL, H. (1977): Über die Cadmiumanreichungen in Champignons. *Z. Lebensmittelunters. u. -Forsch., 164,* 269–271.

MEISCH, H. U., SCHMITT, J. A. & REINLE, W. (1978): Schwermetalle in höheren Pilzen. III. Vanadium und Molybdän. *Z. Neturf., 33,* 1–6.

PIEPPONEN, S., LINKONNEN, L. H. & KUUSI, T. (1983): The selenium content of edible mushrooms in Finland. *Z. Lebensmittelunters. u. -Forsch., 177,* 257–260.

QUINCHE, J. P. (1983): Les teneurs en selenium de 95 especes de champignons superieurs et de quelques terres. *Schweiz. landw. Forsch., 22,* 137–144.

RIMÓCZI, I. (1987): *Az óriás pöfeteg* (Lagermannia gigantea *Batsch.: Pers. (Rostk.) domesztikációjára irányuló biológiai kutatások.* (Biological research on the domestication of *Langermannia gigantea.)* Dissertation, Budapest.

SANTOPRETE, G. & INNOCENTI, G. (1984): Indagani sperimentali sul contenuto di oligoelementi nei funghi del bolognese e di altre provenienze. *Micol. Ital., 1,* 11–28.

SCHMITT, J. A. & MEISCH, H. U. (1985): Cadmium in mushrooms, distribution, growth effects and binding. *Trace Elem. Med., 2,* 163–166.

SEEGER, R. (1978): Cadmium in Pilzen. *Z. Lebensmittelunters. u. -Forsch., 166,* 23–34.

SEEGER, R. & GROSS, M. (1981): Thallium in höheren Pilzen. *Z. Lebensmittelunters. u. -Forsch., 173,* 9–15.

SEEGER, R., MEYER, E. & SCHÖNHUT, S. (1976): Blei in Pilzen. *Z. Lebensmittelunters. u. -Forschung, 162,* 7–10.

SEEGER, R., ORTH, H. & SCHWEINSHAUT, P. (1982): Strontiumvorkommen in Pilzen. *Z. Lebensmittelunters., u. -Forsch., 174,* 381–389.

SOLOMKO, E. F., GRODZINSKAYA, A. A., PASCHENKO, L. A. & PCHELINTSEVA, R. K. (1986): Mineralnij szosztav nekotorih vidov kultivirujemih i dikorastusih gribov klassa Basidiomycetes. *Mikol. Fitopatol., 20,* 474–478.

TYLER, G. (1980): Metals in sporophores of Basidiomycetes. *Trans. Br. mycol. Soc., 74,* 41–49.

TYLER, G. (1982): Metal accumulation by wood-decaying fungi. *Chemosphere, 11,* 1141–1146.

TÖLGYESI, G. & VASS, A. (1984): Bazidiumos nagygombák, valamint magvas növények ásványianyag-tartalmának összehasonlító vizsgálata. (Comparative analysis of the mineral content of Basidial macrofungi and seed bearing plants.) *Agrokém. Talajt., 33,* 125–138.

VASS, A. & TÖLGYESI, G. (1979): Gombák, fák, cserjék, valamint lágyszárú növények ásványianyag-tartalma. (Mineral elements in Fungi, trees, shrubs and soft-stemmed plants.) *Bot. Közl., 66,* 291–298.

VETTER, J. (1987): Magasabbrendű gombák ásványianyag-tartalmának vizsgálata. (Study of the mineral content of higher fungi.) *Mikol. Közl. (Clusiana), 25,* 125–140.

VETTER, J. & HARASZTI, E. (1981): Néhány takarmánynövény stroncium- és cézium-tartalma. (Strontium and cesium content of some fodder crops.) *Bot. Közl., 68,* 205–212.

VETTER, J. & KONECSNI, I. (1981): Egyes ehető gombafajok kémiai összetétele. (Chemical composition of certain edible mushrooms.) *Mikol. Közl., 19,* 87–112.

Acta Alimentaria, Vol. 19 (1), pp. 41—46 (1990)

MINERAL COMPONENTS AND MICRO-ELEMENTS IN JERUSALEM ARTICHOKE TUBERS GROWN IN HUNGARY

J. Barta, P. Fodor, Sz. Török and K. Vukov

Institute of Food Technology, University of Horticulture and Food, H-1118 Budapest, Ménesi út 45. Hungary

The solids, soluble carbohydrate and within this the fructose content, as well as the raw fibre content of Jerusalem artichokes tubers *(Helianthus tuberosus)* grown in Hungary correspond to the average of data found in related literature, while the ash content is somewhat lower.

Ash analysis carried out by IC plasma-spectrometry shows the potassium, magnesium and phosphorus content to agree with the average data found in the literature. The sodium content was found to be slightly lower, while the calcium content is higher than the average. This paper is the first to publish data on the essential micro-element composition of Jerusalem artichoke tubers and the juice pressed from them. Some of the micro-elements can be found partly in the juice (Cu, Cr, Zn), partly they are bound in fiber.

As expected, the micro-element composition in the two samples — grown at two different places — is different.

Keywords: Jerusalem artichoke tubers, mineral components, microelements

The tuber of Jerusalem artichoke *(Helianthus tuberosus)* has been the subject of interest in recent years as an industrial raw material, partly the raw material of alcohol as a source of energy, partly as human food mainly due to its fructosan content (Klaushofer et al., 1987; Fuchs, 1987). The substantial proportion of fructose obtained by hydrolysis from the fructosans is valuable from two standpoints. One of these is in connection with the reduction of sugar consumption: the manufacture of a liquid sugar rich in fructose of lower energy content while producing the same sensation of sweetness and not detrimental to people suffering from diabetes (Barta, 1986; Höhn, 1986). The other trend is the production of a food of high fructose content and at the same time containining all the minerals of the tuber, in particular the micro-elements (Angeli & Bärwald, 1985).

The cultivation of Jerusalem artichoke has advantages for agriculture, too: this plant can be grown with a satisfactory yield on soils where maize, sugar beet or even chicory have very low yields, on loose sour soils, on the soil of woods devastated by acid rains (Molnár, 1986).

Related literature contains data only on the classical food components found in Jerusalem artichokes (Vadas, 1934; Conti, 1953; Kakhana & Arasimovich, 1974; Kaldy et al., 1980; Souci et al., 1981; Kosaric et al., 1984; Bärwald, 1987). Mean values and extreme values of their data are listed in Table 1.

Table 1

Nutrition-biological composition of the solids content of Jerusalem artichokes

Component	Number of data	Average	Minimum	Maximum
Soluble carbohydrate as % of solids (total sugar content)	20	71.6	48.6	80.0
Fructose as % of total sugar	14	82.2	71.8	91.0
Raw protein as % of solids	14	4.2	2.2	9.7
Raw fibre as % of solids	14	4.8	2.4	6.7
Ash content as % of solids	13	6.3	4.2	8.6

As seen in the table the main component of the tubers is their soluble carbohydrate content or reducing sugar content four fifth of which is fructose. Thus, the solids content consists of poly- and oligofructosans. Of the other components ash is the most important fluctuating within wide limits. The majority of available data on the ash components relates to the macro-elements (KALDY et al., 1980; SOUCI et al., 1984). The minerals contained in the solids content of the tubers are listed in Table 2.

Table 2

Ash composition in the solids of Jerusalem artichoke tubers
(mg per 100 g solids)

Component	Number of data	Average	Minimum	Maximum
K	4	2110	1440	2280
Na	3	416	390	431
Mg	4	92	68	110
Ca	3	103	48	134
P	4	159	34	374
Fe	4	21.0	3.3	45.4

As can be seen from the table containing the mean and extreme values a high potassium content, forming about 25–30% of the ash, is characteristic. Data on micro-elements are given only by BÄRWALD (1987). These are as follows: Cu 0.88 mg, Mn 16.7 mg and Zn 2.47 mg in 100 g solids content.

1. Materials and methods

The nutrient composition of Hungarian grown Jerusalem artichoke was obtained by analysing two samples representing the average of 6 varieties grown at the Agrobotanical Center, Tápiószele, County Pest, and picked in

the late autumn of 1986 and 1987 and two other samples, the produce of "New Life" Agricultural Cooperative, Vése, Transdanubia, County Somogy, and harvested in the spring of 1987 and 1988.

The composition of the ash was determined in the tuber samples and in the juice pressed from the tubers grown in Vése and picked in the early spring of 1988 and preserved by quick-freezing. For the sake of comparison the data of a juice pressed from tubers grown in 1986 at Sükösd, County Bács, are also presented.

The total solid content was obtained by drying to constant mass at 105 °C the cut up bits of the tubers. The soluble carbohydrate was determined in the aqueous extract clarified according to Carrez and hydrolyzed with hydrochloric acid for 30 min over a boiling hot water bath by Shoorl's method (Örsi, 1987) as reducing sugar hereinafter named „total sugar content". The fructose content was calculated using Willstätter's jodometric method as modified by Bot (1949) for the determination of glucose and subtracting the result obtained from the total sugar content.

The raw fibre content was determined by the Weende method (Lásztity, 1987). The ash content was obtained by simple incineration. To establish the composition of the ash a Jarrel-Ash 61 type IC plasma-spectroscope was used.

From the homogenized samples 1.00 g was weighed in sealable teflon vessels of 100 cm^3 volume. Two cm^3 concentrated nitric acid and 2 cm^3 30% hydrogenperoxide were added, then in a closed system the organic matter was oxidized under slight overpressure at 100 °C for 1 h. The clear solutions were filled up to 10 cm^3 with ion-exchanged distilled water and the samples were transferred by way of a peristaltic feeder to the Babington-system atomizer of the plasma-spectrometer.

The instrument was optimized to the lowest trouble level applying 14 dm^3 per min cooling gas flow, 16 mm height and a capacity of 1.05 kW. The apparatus was calibrated with a synthetic model solution. Measurements were carried out with 7 s integration period, by the aid of double-sided background correction. Only the true sign was taken into account. The spectra of the disturbing lines were built in the measuring program, thus, the effect of the coincidence of the lines was minimalized.

Data were evaluated statistically on an Apple II e computer.

2. Results

The composition of the Jerusalem artichoke tubers grown in Hungary can be seen in Table 3.

The mineral components determined are listed in Table 4.

The composition of the tubers grown on sandy soil at Vése, County Somogy, and of the juice pressed from the tubers, are shown separately. The

Table 3

Composition of Hungarian Jerusalem artichoke tubers

	Average	Standard deviation
Total solids content (%)	21.0	±4.4
Soluble carbohydrate (total sugar) as % of solids	70.5	±6.3
Fructose as % total sugar	77.6	±5.5
Raw fibre as % of solids	4.1	±0.2
Ash as % of solids	3.75	±0.13

Table 4

Composition of the solids of the tubers and juice of Jerusalem artichokes

Elements	Sandy soil in County Somogy (Vése)				Sandy soil in County Bács (Sükösd) Juice
	Tubers		Juice		
	average	standard deviation	average	standard deviation	average
K	2150	±58	1950	±144	1340
Na	21	± 0.9	15	± 3	60
Mg	97	±13	59	± 13	58
Ca	220	± 7.9	118	± 25	78
P	343	± 7.5	220	± 32	212
Fe	4.2	± 0.17	7.4	± 2.5	0.9
Cu	2.1	± 1.4	0.59	± 0.15	0.29
Cr	0.11	± 0.02	0.03	± 0.05	0.07
Mn	1.12	± 0.05	3.63	± 2.01	1.01
Zn	2.93	± 0.61	1.62	± 1.09	0.91
Ti	0.02	± 0.01	0.04	± 0.03	0.01

first shows the result of the analysis of a single sample in three parallels. Under the pressed juice heading the averages and standard deviations of the analyses of the four juice samples obtained by pressing the tubers picked from the same plot at four different times, are given. Under the third heading the analytical data of the juice pressed under industrial conditions from tubers grown on the sandy soil of County Bács, are presented. Standard deviations were not calculated. Apart from the essential micro-elements as listed in Table 4 the following were found in 100 g solids of Jerusalem artichoke tubers: Si 4.2 mg, Al, Ba, Sr cca 1 mg, Ni cca 0.3 mg, Co, Mo, Cd and Plb 0.01–0.02 mg and finally Se, As and Hg in amounts not determinable.

3. Conclusions

By comparing data in Table 3 as determined in Hungary with those found in related literature (Table 1) it can be seen that the soluble carbohydrate content of solids and the proportion of fructose approximates the averages of foreign data. The same is valid for the raw fibre content, however, the ash content of Hungarian samples is definitely lower than the data given in the literature. As seen in Table 4, presenting the micro-element composition of ash as measured in Hungary, the potassium content corresponds to related data in the literature while the sodium content is substantially lower. Magnesium and phosphorus correspond, too, while the calcium content is higher than the data found. Data found on the iron content vary within a wide range and may originate occasionally from iron utensils used to cut up the tubers. Values measured in Hungary are higher than the minimum as found in the literature.

Although the juice samples analyzed were not prepared from the tubers analyzed, they originated from the same plot in County Somogy, therefore some conclusions may be drawn as to the proportion of elements extracted by pressing from the tubers (Table 4). Alkali metals (K, Na) get transferred practically quantitatively into the juice. From the earth metals (Ca, Mg) and phosphorus about two third of the original amount gets into juice. The iron content of the juice fluctuates substantially and shows an increasing tendency in comparison to the tubers, indicating the presence of iron brought in solution from the utensils used. A similar tendency is shown also by the manganese content. Copper, chromium and zinc, however, get transferred only partly into the juice in the course of pressing. According to the aforesaid, a certain part of the following elements is bound to the fibres of the artichoke tubers: alkali earth metals (Ca, Mg), phosphorus, copper, chromium and zinc. In spite of this the juice pressed from Jerusalem artichokes tubers is rich in potassium as well as in essential microelements.

However, the values measured vary to a certain extent as a function of the place of cultivation.

Literature

ANGELI, I. & BÄRWALD, G. (1985): Vorläufige Ergebnisse über die Verwendung von Topinambursaft in Diät und bei Diabetikern. *Gordian, 85,* 239–244.

BARTA, J. (1986): Fruktóz előállítása csicsókából. (Fructose production from Jerusalem artichokes.) *Élelm. Ipar, 40,* 465–469.

BÄRWALD, G. (1987): Über die Verarbeitung von Topinamburknollen zu verschiedenen Producten der Lebensmittelindustrie. *Renaissance der Topinambur, Siófok 1987.* FTTE, Budapest. pp. 91–96.

BOT, GY. (1949): A cukrok jodometriás mérése. (Jodometric measurement of sugars.) *Magy. Kém. Lap., 4,* 291–294.

CONTI, F. W. (1953): Die Gewinnung der Kohlenhydrate der Topinambur. *Zucker, 6,* 120–125.

FUCHS, A. (1987): Das Interesse für Topinambur in den Niederlanden. *Renaissance der Topinambur, Siófok 1987.* FTTE, Budapest, pp. 44–48.

HÖHN, E. (1986): Topinambur — eine süsse Geschichte ? *Lebensmittel Technol., 19,* (2), 32–36.

KAKHANA, B. M. & ARASIMOVICH, V. V. (1974): *Biokhimiya topinambura.* Akademiya nauk Moldavskoj SSR, Institut Fiziologii Biokhimija rasstenij, Kishinev, p. 86.

KALDY, M. S., JOHNSTON, A. & WILSON, D. B. (1980): Nutritive value of Indian breadroot, squaw-root and Jerusalem artichoke. *Econ. Bot., 34,* 352–357.

KLAUSHOFER, H., ABRAHAM, B. & SCHWAIGER, I. (1987): Topinamburforschung in Österreich. *Renaissance der Topinambur, Siófok 1987.* FTTE, Budapest, pp. 25–39.

KOSARIC, N., COSETINO, G. P., WIECZOREK, A. & DUVNJAK, Z. (1984): The Jerusalem artichoke as an agricultural crop. *Biomass, 5,* 1–36.

LÁSZTITY, R. (1987): Gabonatermékek vizsgálata. (Analysis of cereals.) – in: *Élelmiszeranalitika II. köt.* (Food analysis, Vol. 2.) Mezőgazdasági Kiadó, Budapest, pp. 21–115.

MOLNÁR, L. (1985): A csicsóka termesztése és hasznosítása. (Cultivation and utilization of Jerusalem artichokes.) MÉM — Bárdibükki Á. G., Bárdudvarnok. pp. 7–16; 43–64.

ÖRSI, F. (1987): Cukor- és édesipari termékek vizsgálata. (Analysis of sugar and confectionery products.) – in: *Élelmiszeranalitika II.* (Food analysis. Vol. 2.) Mezőgazdasági Kiadó, Budapest, pp. 115–160.

SOUCI, S. W., FACHMANN, W. & KRAUT, H. (1984): *Die Zusammensetzung der Lebensmittel. Nahrwert-Tabellen 1981/82.* Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp. 828–829.

VADAS, R. (1934): Technische Gewinnung von Alcohol aus Topinambur. *Chemikerzeitung, 58,* 249–250.

Acta Alimentaria, Vol. 19 (1), pp. 47—53 (1990)

DETERMINATION OF FREE AMINO ACID CONTENT OF VARIETAL RED WINES FROM THE TARRAGONA REGION. A STUDY OF THE VARIETAL INFLUENCE

M. CALULL, R. M. MARCÉ, J. GUASCH and F. BORRULL

Department de Química, Facultat de Ciències Químiques, Universitat de Barcelona, Pça. Imperial Tarraco No 1, 43005 Tarragona. Spain

(Received: 20 March 1989; accepted: 30 June 1989)

The free amino acid content of different varietal red wines has been determined by HPLC. The varieties studied were: Garnatxa, Carinyena, Ull de Llebre, Cabernet Sauvignon and Merlot, all of which are cultivated in four experimental vineyards in the Tarragona region. The first three varieties are traditionally cultivated in Catalonia, and the last two have been recently introduced into this country to enhance the quality of its red wines. The results show an influence of each of these varieties on the amino acid content, with higher levels for the varieties Cabernet Sauvignon and Merlot. The results obtained have been related to two enological parameters, the ethanol and the total acidity content.

Keywords: wine, amino acids, HPLC

In this paper, the free amino acid content in varietal red wines from the Tarragona region is reported. In red wines the amino acid content has an important influence on the organoleptic properties of the wine after its aging, as a result of its combination with sugars to form Maillard compounds.

It is obvious that a relation exists between the variety and the content of amino acids. For this reason, new varieties have been introduced in this region to improve the quality of the wines.

The most usual method of determining amino acids in wine is the OPA method (SANDERS et al., 1985; ISHIDA et al., 1981; PFIEFER et al., 1983) but in this paper we have used a modification of the PICO-TAG method, developed by Waters (BIDLINGMEYER et al., 1984), and previously optimized for its application to wine analysis in another paper (MARCÉ et al., 1989). This method allows the determination of proline and hidroxyproline which can not be determined by OPA method. The difference in concentration between the proline (1000–2000 ppm) and the rest of the amino acids (less than 10 ppm) has not been an obstacle in applying this method.

On the other hand, in this study the content of amino acids in varietal wines is related to two enological parameters: the acidity and the ethanol content. A statistical study between these two parameters has been carried out for the varieties studied.

1. Materials and methods

1.1. Samples

Wines obtained from five different varieties were studied. Three of these varieties (Garnatxa, Carinyena and Ull de Llebre) are traditionally cultivated in Catalonia to obtain the typical wines with D. O. Terra alta, Tarragona and Conca de Barberà. The other two varieties (Cabernet Sauvignon and Merlot), which have not been traditionally cultivated in this country have been recently introduced to enhance the quality of its red wines. All these wines have been provided by INCAVI (Institut Català de la Vinya i del Vi) and they have been cultivated in experimental vineyards.

For this study, 18 varietal red wines were selected: 4 Cabernet-Sauvignon wines, 4 Merlot wines, 4 Ull de Llebre wines, 3 Carinyena wines and 3 Garnatxa wines, from 4 experimental vineyards of INCAVI. These experimental vineyards are located in Batea (D. O. Terra Alta), Bràfim (D. O. Tarragona) and Barberà (D. O. Conca de Barberà) in the Tarragona region and Verdú, located in the northern part of this region. A wine sample of each variety was obtained from each vineyard, except from the Bràfim vineyard where the varieties Garnatxa and Carinyena are not cultivated.

Before the derivatization step, all wine samples were cleaned by using a micropartition system to eliminate the interferences in the chromatogram (MARCÉ et al., 1989).

1.2. Derivatization procedure

The analysis of amino acids was carried out by derivatization with phenylisothiocyanate (PITC) and chromatographic determination by HPLC, following the method previously reported (MARCÉ et al., 1989). For this work, the PTC-derivatives have been chosen because they offer some advantages (analysis time, use of the UV detector, stability of the derivatives, etc.) over and above the other methods. This method enables us to analyze the 22 most important amino acids in 35 min.

The following reagents were used in the dervatization step: PITC (phenylisothiocianate), water, ethanol and trietylamine in the ratio 1 : 1 : 7 : 1 (HEINRIKSON et al., 1984; BIDLINGMEYER et al., 1984; CHAO-YUH YANG et al., 1985; SCHOLZE, 1985). A 60 μl volume of wine, previously cleaned, was evaporated to dryness under a high efficiency vacuum pump, equipped with a cold trap, and 20 μl of derivative solution were added to the residue. After a 20 minute reaction at room temperature, the reagents were removed under vacuum conditions. The residue was dissolved in 100 μl of acetonitrile–water (2 : 7) and the solution was immediately chromatographed.

1.3. Chromatographic conditions

The separation was carried out on a Hewlett-Packard 1090 chromatograph using a Spherisorb ODS column (25×0.46 cm i.d.) with 5 μm particle size and a Bondapack C18 Corasil (37–50 μm particle size) precolumn. The injection system consisted of a Rheodyne loop of 5 μl. The flow rate was 1 cm^3 min^{-1} and the temperature was constant at 52 °C. The derivatized amino acids were detected at 254 nm.

The mobile phase consisted of three different solvents: a) 0.05 mol l^{-1} NaAc with 0.25% acetonitrile. b) 0.1 mol l^{-1} NaAc and acetonitrile 50 : 50 and c) acetonitrile and water 70 : 30. The pH of the solution "a" and "b" was adjusted to pH 6.8 with acetic acid. The lineal multi-step solvent gradient used was reported in a previous paper (MARCÉ et al., 1989).

2. Results and discussion

Table 1 shows the different varietal wines, their origin and the values of the following enological parameters: Ethanol (% v/v), total acidity (g dm^{-3} as tartaric acid) and pH. It is interesting to observe the difference between

Table 1

Enological parameters of varietal wines

Variety	Origin	Ethanol (% v/v)	Total acidity (g dm^{-3} tartaric acid)	pH
Garnatxa	Batea	10.55	6.80	3.2
	Barberà	9.30	11.25	3.0
	Verdú	11.90	6.15	3.4
Ull de Llebre	Batea	10.00	5.90	3.6
	Barberá	10.70	7.70	3.4
	Verdú	12.05	5.45	3.7
	Bràfim	9.30	7.20	3.4
Carinyena	Batea	11.40	6.90	3.5
	Barberà	8.80	10.70	3.0
	Verdú	10.95	5.60	3.5
Cabernet Sauvignon	Batea	11.40	6.60	3.3
	Barberà	10.55	9.90	3.1
	Verdú	12.45	5.90	3.5
	Bràfim	9.30	8.70	3.1
Merlot	Batea	13.00	6.05	3.3
	Barberà	11.75	7.35	3.2
	Verdú	13.20	5.70	3.6
	Bràfim	11.35	6.00	3.4

the percentage of ethanol of the wines. The wines from Verdú and Batea showed higher values of alcohol content than the Barberà and Brafim ones. A statistical study of these values showed a significant difference (F = 12.54) between the values of these wineyards. The differences were maximized if the values were grouped in two groups: the Batea-Verdú and the Barberà-Bràfim.

Value F has been calculated by the expression:

$$F = S_1^2/S_2^2$$

where S_1 and S_2 are the standard deviation, the first one between the different groups and second into each group. The expression to calculate S are:

$$S = SS/(\text{degrees of freedom.})$$

(1) SS_1 between the different groups are:

$$SS_1 = \frac{(\Sigma y)^2}{n_i} - \frac{(\Sigma\Sigma y)^2}{\Sigma n_i}$$

(2) SS_2 in each group are:

$$SS_2 = SS_{Total} - SS_1$$

$$SS_{total} = \Sigma\Sigma y^2 - \frac{(\Sigma\Sigma y)^2}{\Sigma n_i}$$

The F value obtained is compared with the F value tabulated (SOKAL et al., 1968).

In the study of the total acidity, it can be observed that the acidity of the Barberà wines is higher than the rest of the wines, but the statistical study showed that the most significant differences can be found (F = 16.67) if the same two groups are defined: the Barberà-Bràfim and Batea-Verdú.

No significant differences can be observed if the percentage of ethanol and the total acidity are studied in function of the variety.

These enological parameters (ethanol, total acidity and pH) can be related to the climatic and soil characteristics. The Barberà and the Bràfim vineyards are located in neightbouring zones and they have analogous climatic conditions. The Verdú and the Batea vineyards are not geographically close to each other, but their climatic conditions are practically the same, with drier weather and higher temperatures during the vegetative period.

Table 2 shows the amino acid distribution in the varietal wines studied here. Firstly, very clear influences among the amino acid content of the varietal wines were not observed. However it is possible to see a higher concentration of amino acids in the Batea and Verdú wines as opposed to the Bràfim and Barberà ones. This relation is similar to that obtained when enological parameters are compared. In this study when varieties in each region are compared a higher concentration of the amino acids of the foreign

Table 2

Amino acid content (mg dm^{-3}) *of varietal wines*

	Carinyena		Garnatxa		Ull de llebre		Cabernet S.		Merlot	
	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max	$\bar{x}$	min-max
ASP	5.0	4.0—6.2	5.1	3.1—7.9	4.2	1.9—6.4	1.6	0.6—3.5	5.4	3.6—8.7
GLU	4.7	2.4—7.8	4.1	0.6—7.1	4.6	3.2—5.6	2.4	0.6—4.5	—	<0.5—7.6
OH-PR	1.2	0.9—1.8	4.6	3.0—5.7	—	<0.5—4.2	12.1	7.9—15.1	5.8	4.3—9.2
SER	3.8	2.5—6.0	5.0	2.5—7.7	3.4	1.8—5.0	2.4	1.2—3.3	3.6	2.5—5.1
ASN	2.4	2.4—2.5	6.3	4.2—7.5	3.3	2.0—5.0	5.6	3.8—8.4	4.2	2.2—7.5
GLY	4.6	2.7—7.1	5.8	3.6—8.5	4.7	2.9—6.0	2.9	1.7—3.6	4.7	3.6—6.3
GLN	ND		ND		ND		ND		ND	
CIT	ND		ND		ND		ND		ND	
THR	5.9	5.1—6.9	5.7	4.6—6.8	4.2	3.3—5.7	4.8	2.5—5.8	5.7	2.1—9.1
ALA	4.8	3.5—7.3	6.4	3.5—9.8	6.5	4.9—8.1	2.6	2.0—3.1	4.1	2.7—6.7
ARG	—	ND —2.6	4.1	1.5—7.2	—	ND —1.1	—	ND —3.0	—	<0.5—1.61
PRO	379	155—579	756	436—993	406	211—711	1618	978—2761	1281	221—197
TYR	2.4	1.5—3.9	4.5	1.9—6.8	2.7	1.3—4.7	2.4	1.0—3.6	3.1	1.9—4.5
VAL	2.1	1.7—2.8	2.7	1.1—3.8	2.6	1.4—4.0	1.3	1.1—1.8	1.4	0.8—2.7
MET	—	<0.5—1.0	2.2	1.3—3.6	—	<0.5—1.5	—	<0.5—1.4	—	<0.5—0.7
ILE	2.5	1.4—3.6	—	ND —3.1	3.0	1.8—4.0	1.6	1.1—2.2	—	<0.5—333
LEU	3.4	2.6—5.1	4.0	1.4—6.3	3.1	1.3—6.6	1.0	0.7—1.6	1.9	0.6—4.2
PHE	4.4	3.6—5.8	8.1	5.2—12.1	4.2	2.8—6.4	2.9	2.5—3.6	5.1	2.8—7.
TRP	ND		ND		ND		ND		ND	
ORN	2.0	1.0—3.2	—	ND —5.4	3.0	1.8—4.0	1.7	1.1—2.3	2.4	0.6—4.7
LYS	5.4	3.8—7.5	4.9	2.3—9.2	6.0	3.3—10.5	2.2	1.7—3.1	3.7	2.4—6.4
AA_{Tot}		433.6		829.5		461.5		1665.5		1332.1
AA_{Tot}–PRO		54.6		73.5		55.5		47.5		51.1

ND: peak not detected
$AA_{Tot.}$: mean of the total amino acid concentration
$AA_{Tot.}$–PRO: mean of the total concentration of amino acids except proline]

varieties in front of the native varieties can be observed for Bràfim, Batea and Verdú wines. In Barberà wines although the concentration of amino acids in the Cabernet Sauvignon variety is higher than the other one, the concentration of amino acids in Merlot variety is smaller in this case.

It can be noted that, although the proline concentration varies in different wines (max. 2761 ppm of Cabernet Sauvignon from Verdú and min. 155 ppm of Garnatxa from Batea), the concentration of the rest of the amino acids is very low for all the wines studied (max. 103 ppm of Carinyena from Verdú and min. 41 ppm of Merlot from Batea or Barberà, and Cabernet Sauvignon from Bràfim), and each amino acid concentration is lower than 10 ppm.

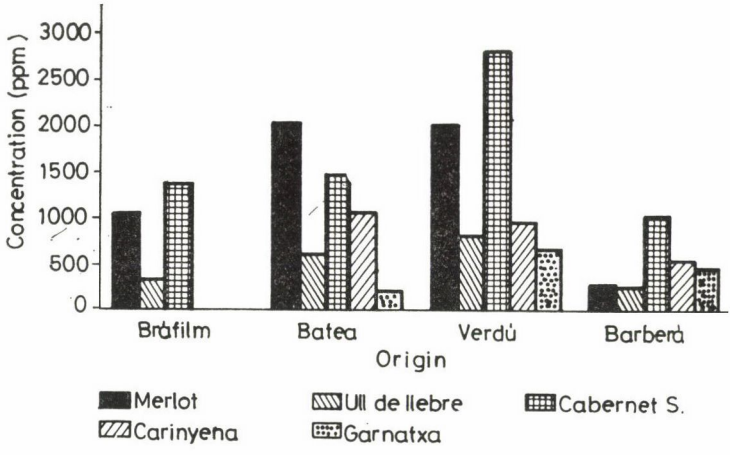


Fig. 1. Distribution of the amino acid content

Figure 1 shows these results graphically. The higher content of amino acids in foreign varieties (Merlot and Cabernet Sauvignon) in all the vineyards must be emphasized except for Merlot variety in Barberà. This higher content is mainly due to the high content of proline as can be seen in Table 2. When the results are analysed, we can conclude that the Verdú wines have the highest concentration of amino acids for every variety and the lowest concentration of amino acids corresponds to the Barberà wines. This means that the concentration of amino acids is also influenced by the vineyard conditions.

If we relate the concentration of amino acids to the percentage of ethanol, it can be seen that the samples with a higher concentration of amino acids have higher ethanol percentage and a lower total acidity.

3. Conclusions

A general conclusion can not be drawn, since only wines of the 1985 vintage have been studied. However, it is important to emphasize the low value of the amino acids content of these wines in contrast to other Spanish (AMERINE et al., 1976; CACERES et al., 1986), French (LHUGUENOT et al., 1979; COLAGRANDE et al., 1984) or Italian wines (BERTOLINI et al., 1976). The range of the proline concentration is considerably wide (from 210 to 2700 ppm) as in French wines, for example. But it is important to emphasize that in some French wines the concentration of any other amino acid is higher than 100 ppm. For example, the alanine is higher than 100 ppm for four wines of different regions: Beaujolais, Viré, Bordeaus and Assace (LHUGUENOT et al., 1979).

Also both the aspartic acid–asparagine and glutamic acid–glutamine are higher in these wines.

*

The authors wish to thank INCAVI for the samples supplied and the CICYT (project No PA 86-0029) and CIRIT (project AR 86-111) for their financial support.

Literature

AMERINE, M. A. & OUGH, C. S. (1976): *Análisis de vinos y mostos.* Wiley, New York.

BERTOLINI, C. & PARONETTO, L. (1976): Variazioni del contenuto in prolina del vino *Vignevini, 3,* 13–18.

BIDLINGMEYER, B. A., COHEN, S. A. & TARVIN, T. L. (1984): Rapid analysis of amino acids using pre-column derivatization. *J. Chroma., 336,* 93–104.

CACERES, I., BARAHONA, F. & POLO, C. (1986): El análisis integro de los vinos. IV. Cromatografia de líquidos de alta eficacia. *Alimentación, equipos y tecnologia,* 141–152.

CHAO-YUH YANG & SEPULVEDA, F. I. (1985): Separation of phenylthiocarbamyl amino acids by high-performance liquid chromatography on Spherisorb octadecylsilane columns. *J. Chromat., 346,* 413–416.

COLAGRANDE, O., SILVA, A. & CASOLI, A. (1984): Acides aminés dans les vins mousseux. *Connaissance Vigne Vin, 18,* 27–48.

HEINRIKSON, R. L. & MEREDITH, S. C. (1984): Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Analyt. Biochem., 136,* 65–74.

ISHIDA, Y., FUJITA, T. & ASAI, K. (1981): New detection and separation method for amino acids by high-performance liquid chromatography. *J. Chromat., 204,* 143–148.

LHUGUENOT, J. C., UDE, L., DYMARSKI, E. & BARON, C. (1979): Analyse des acides aminés libres dans les vins et dans les mouts de raisin en cours de fermentation alcoolique par chromatographie gaz-liquide. *Annals Falsif. Expert. chim., 72,* 275–286.

MARCÉ, R. M., CALULL, M., GUASCH, J. & BORRULL, F. (1989): Determination of free amino acids in wine by HPLC using precolumn derivatization with phenylisothiocyanate *Am. J. Enol. Vitic., 40,* No. 3. 194—198.

PFIEFER, R., KAROL, R., KERPI, J., BURGOYNE, R. & MCCOURT, D. (1983): *Practical application of HPLC to amino acid analysis.* American Laboratory (March).

SANDERS, E. M. & OUGH, C. S. (1985): Determination of three amino acids in wine by HPLC. *Am. J. Enol. Vitic., 36,* No. 1. 43–46.

SCHOLZE, H. (1985): Determination of phenylthiocarbamyl amino acids by reversed-phase high-performance liquid chromatography. *J. Chromat., 350,* 453–460.

SOKAL, R. R. & ROHLF, F. J. (1968): Biometria, principios y métodos estadisticos en la investigación biológica. H. Blume, Madrid.

Acta Alimentaria, Vol. 19 (1), pp. 55–61 (1990)

ENZYMATIC AND TOTAL AMINO ACID CHANGES UNDER DIFFERENT STORAGE CONDITIONS FOR DAMAGED AND UNDAMAGED BEETS[a]

P. Spettoli,[b] A. Curioni,[b] A. Crapisi,[b] G. Vaccari,[c] and G. Mantovani[c]

[b]Dipartimento di Biotecnologie Agrarie, Università di Padova,
via Gradenigo 6, 35131 Padova. Italy
[c] Dipartimento di Chimica, Universita di Ferrara,
via Borsari 46, 44100 Ferrara. Italy

(Received: 10 April 1989; accepted: 21 June 1989)

Damage to beet roots is a source of sugar loss, either by the formation of fragments which cannot be recovered or by metabolic and enzymatic processes particularly active during the first hours following the damage. Sucrose decomposition and its regulation within practical beet conditions are still not completely understood. Acid and neutral invertase and sucrose synthetase, key enzymes of carbon metabolism in plants, aminopeptidase, splitting off an N-terminal residue with a free amino group, and total amino acid content in damaged and undamaged sugarbeets, were evaluated. Enzymatic and amino acid analyses on the cold and hot extraction juice of the "brei" respectively were carried out. Beet samples of damaged vs. undamaged, interior vs. surface of pile, factory silos vs. field pile, T_2 vs. T_0, T_4 vs T_2 and T_4 vs. T_0 days by a one way analysis of variance and orthogonal contrasts showed significantly higher specific enzyme activity values ($P \leq 0.01$). The total amino acid content assayed in hot extraction juice and particularly aspartic and glutamic acid, serine and glycine levels were subject to slight variations.

Keywords: amino acids, beets, enzymes, storage

The evaluation of the injury of sugarbeet roots during harvesting and handling, in relation to their technological quality decrease during storage prior to sugar factory operations, has been extensively investigated (Akeson & Stout, 1978; Baraldi et al., 1983; Cole, 1977; Mc Cready & Goodwin, 1966; Peterson et al., 1980; Wyse, 1978; Wyse & Dexter, 1971; Wyse & Peterson, 1979). Damage to beet roots is a substantial source of sugar loss, either by the formation of fragments which cannot be recovered or by metabolic and enzymatic processes particularly active during the first hours following the damage (Vaccari et al., 1988).

The sucrose decomposition and its regulation within practical beet storage conditions are still not completely understood (Vukov & Hangyál, 1985). Therefore our purpose was to evaluate acid and neutral invertase and sucrose synthetase, key enzymes of carbon metabolism in plants, aminopeptidase, splitting off an N-terminal residue with a free amino group, and total amino acid content in damaged and undamaged sugarbeets.

[a] Presented at 25th General Meeting, American Society of Sugar Beet Technologists, February 26–March 2, 1989, New Orleans La. U.S.A.

1. Materials and methods

A pile of about 10 tons of mechanically harvested roots was made up. Six samples of damaged and six of undamaged beets of about 150 kg of roots were taken out from the surface and the interior of the pile and analyzed in the sequence shown in Fig. 1, peak storage time of four days.

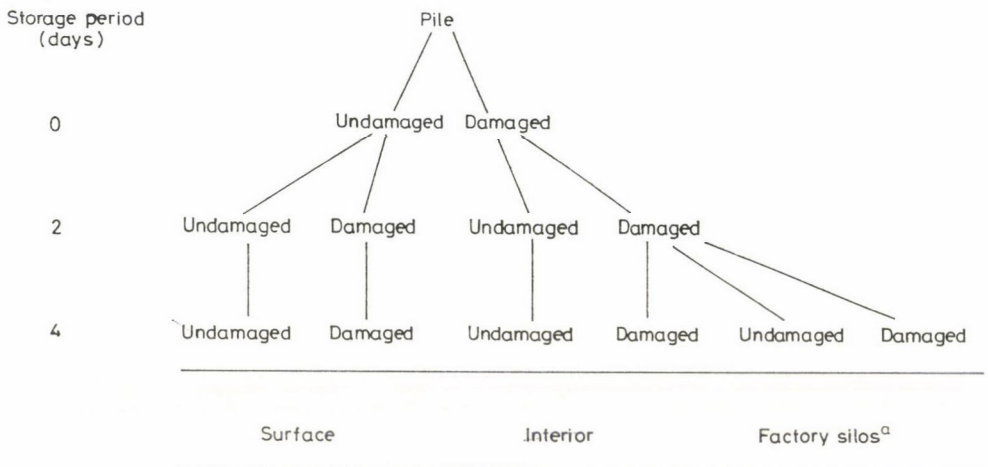


Fig. 1. Sampling scheme at different storage times and conditions for damaged and undamaged beets
[a] The sugar beets were taken from the interior of the pile after a storage period of two days

The dry substance and polarization analyses were carried out in the "brei". The enzymatic investigation was made on the extraction juice of the same "brei", stored at −20 °C. Total amino acid content (Amino Acid Analyzer Beckman 118 CL) and reducing sugars (% sucrose) were assayed in juice obtained with water at 80 °C from the "brei" to simulate the raw juice found in sugar factory.

The specific activities of acid and neutral invertase (EC 3.2.1.26) and sucrose synthetase (EC 2.4.1.13) were determined as previously reported (VACCARI et al., 1988). The activity of aminopeptidase (EC 3.4.11.1) was evaluated as described in the literature reference (DESMAZEAUD & JUGE, 1976). Protein determination was carried out following the Bradford method (BRADFORD, 1976). Minimum values for temperature during beet root storage ranged between 19–21 °C and the maximum between 32–34 °C; the minimum for the relative humidity varied between 58–70% and the maximum between 85–97%.

2. Results

The values of all specific enzyme activities increased significantly ($P \leq 0.05$) with storage time as shown in Table 1. Sucrose synthetase, acid and neutral invertase levels had risen to about 2–3 times the initial values after a storage period of four days. These data are in accordance with data of Hungarian researchers who found a similar trend for invertase activity in beet stored in large-scale piles but at the end of a sixty-day period (VUKOV & HANGYÁL, 1985), and with WYSE (1974), BURBA & NITZSCHKE (1980), and with our previous studies (BARALDI et al., 1984; BENTINI et al., 1987; VACCARI et al., 1983; VACCARI et al., 1988). Temperature and climatic conditions are the reason for similar results among different storage periods. The increase of the above mentioned enzyme activities could be responsible for the increase of reducing sugars (% sucrose) and the counteracting decrease of polarization (% dry substance) observed in damaged and undamaged beet roots after four day storage (Table 2).

Table 1

Changes in enzyme activities at different storage times and conditions for damaged and undamaged beets. The activities of acid and neutral invertase and sucrose synthetase are expressed in terms of μmoles of sucrose hydrolised 1/2 h^{-1} mg^{-1} protein. The activity of aminopeptidase in terms of Δ O. D. 1/2 h^{-1} mg^{-1} protein

Storage period (days)	Pile	Beet root condition	Sucrose synthetase	Acid invertase	Neutral invertase	Amino peptidase
0		undamaged	1.39^{A}	2.79^{A}	2.83^{A}	2.49^{AB}
		damaged	1.53^{A}	3.26^{B}	3.60^{B}	2.42^{AB}
2	surface	undamaged	1.80^{B}	4.24^{C}	4.50^{C}	2.26^{A}
		damaged	1.93^{B}	4.49^{C}	5.46^{D}	2.55^{B}
	interior	undamaged	1.81^{B}	4.31^{C}	4.53^{C}	2.69^{B}
		damaged	2.29^{C}	6.29^{D}	6.06^{E}	3.38^{D}
4	surface	undamaged	2.67^{D}	7.49^{E}	6.75^{F}	3.00^{C}
		damaged	2.55^{D}	8.68^{F}	8.41^{H}	3.44^{D}
	interior	undamaged	2.81^{E}	7.65^{E}	6.56^{F}	3.36^{D}
		damaged	3.47^{F}	10.52^{G}	9.67^{I}	3.93^{E}
	factory silos[a]	undamaged	1.90^{F}	6.63^{D}	6.44^{EF}	3.06^{C}
		damaged	3.61^{F}	8.29^{F}	7.87^{G}	3.92^{E}
	Residual standard deviation		0.0174	0.0802	0.0708	0.0210
	Degrees of freedom		34	34	34	34

$^{A, B, C}$ Means in the same column with unlike subscript differ at $P \leq 0.05$ probability level

[a] The sugar beets were from the interior of the pile after a storage period of two days. Differences were measured by a one-way analysis of variance and Neuman-Keuls Test

Table 2

Variation of reducing sugars and sucrose at different storage times and conditions for damaged and undamaged beets

Storage period (days)	Pile	Beet root	Reducing sugars (% sucrose)	Polarization (% d. s.)
0	—	undamaged	1.39	76.61
		damaged	1.66	76.25
2	surface	undamaged	3.22	73.61
		damaged	3.26	71.67
	interior	undamaged	2.39	75.06
		damaged	2.97	72.04
4	surface	undamaged	4.69	73.34
		damaged	4.21	69.57
	interior	undamaged	3.26	73.62
		damaged	4.68	70.60
	factory silos[a]	undamaged	4.74	69.79
		damaged	5.29	68.00

[a] The sugar beets were taken from the interior of the pile after a storage period of two days

The aminopeptidase increase could be partially in accordance with VUKOV & HANGYÁL (1985) who reported that a considerable amount of protein hydrolysis occurred in damaged beet batches. On the contrary DEXTER and co-workers (1966) described an amino acid decrease in sugarbeets stored at 3 °C apparently as a result of the synthesis of new enzymes and other proteins, in agreement with WYSE & DEXTER (1971). Moreover beet samples of damaged vs. undamaged, interior vs. surface of pile, factory silos vs. field pile,

Table 3

Influence of damage, surface and interior of pile, factory silos and storage period on enzyme activities of beets

Main effects	Contrasts	Sucrose synthetase	Acid invertase	Neutral invertase	Amino peptidase
Beet root condition	undamaged-damaged	—0.48**	—1.38**	—1.56**	—0.46**
Field pile	surface-interior	—0.36**	—0.97**	—0.43**	—0.53**
Beet storage method	field pile-factory silos	—0.34*	—0.75**	—0.66**	—0.41**
Storage period (days)	0—2	—0.50**	—1.81**	—1.92**	—0.27**
	0—4	—1.42**	—5.56**	—4.63**	—0.98**
	2—4	—0.92**	—3.75**	—2.71**	—0.71**

* Significant at $P \leq 0.05\%$ probability level
** Highly significant at $P \leq 0.01\%$ probability level
Differences were measured by a one may analysis of variance and orthogonal contrasts

Table 4

Total amino acid content at different storage times and conditions for damaged and undamaged beets (g % brix)

Storage period (days)	Pile	Beet root condition	ASP.	THR.	SER.	GLU.	GLY.	ALA.	VAL.	MET.	ILE.	LEU.	TYR.	PHE.	HIS.	LYS.	ARG.	Total
0	—	undamaged	0.86	0.15	0.46	3.12	0.12	0.68	0.20	0.07	0.24	0.27	0.16	0.12	0.09	0.15	0.14	6.83
		damaged	0.86	0.15	0.41	2.18	0.14	0.62	0.20	0 07	0.26	0.25	0.16	0.10	0.09	0.15	0.14	5.78
2	surface	undamaged	0.48	0.12	0.29	2.09	0.15	0.47	0.18	0.07	0.21	0.24	0.09	0.12	0.09	0.15	0.14	4.89
		damaged	0.65	0.15	0.25	1.57	0.11	0.47	0.19	0.06	0.20	0.22	0.11	0.12	0.11	0.15	0.14	4.50
	interior	undamaged	0.53	0.11	0.37	2.90	0.10	0.56	0.19	0.07	0.21	0.22	0.11	0.10	0.14	0.12	0.16	5.89
		damaged	0.54	0.15	0.42	2.78	0.14	0.55	0.15	0.09	0.21	0.24	0.11	0.12	0.09	0.13	0.16	5.88
4	surface	undamaged	0.53	0.14	0.35	2.31	0.10	0.54	0.22	0.15	0.22	0.22	0.07	0.12	0.11	0.13	0.16	5.37
		damaged	0.62	0.17	0.34	1.74	0.13	0.53	0.18	0.06	0.22	0.21	0.09	0.08	0.09	0.15	0.14	4.75
	interior	undamaged	0.78	0.15	0.41	2.19	0.10	0.59	0.19	0.07	0.21	0.25	0.07	0.10	0.09	0.13	0.12	5.45
		damaged	0.82	0.14	0.32	1.75	0.10	0.55	0.18	0.07	0.21	0.24	0.07	0.12	0.09	0.13	0.12	4.91
	factory silos[a]	undamaged	0.77	0.17	0.45	2.94	0.15	0.60	0.20	0.07	0.22	0.24	0.05	0.10	0.09	0.16	0.14	6.35
		damaged	0.62	0.12	0.32	2.76	0.10	0.49	0.18	0.09	0.17	0.20	0.07	0.08	0.08	0.10	0.14	5.52

[a] The sugar beets were taken from the interior of the pile after a storage period of two days

T_2 vs. T_0, T_4 vs. T_2 and T_4 vs. T_0 days by a one way analysis of variance and orthogonal contrasts showed significantly higher specific enzyme activity values ($P \leq 0.01$) (Table 3). The total amino acid content found in hot extraction juice and particularly aspartic and glutamic acid, serine and glycine and glycine levels were subject to slight variations (Table 4). No relation was observed comparing the values of aminopeptidase activity and total amino acid amounts since the total amino acid levels did not necessarily come only from proteolytic enzymes but also from other factors related to the hot extraction process and connected i.e. with the changes of beet tissue conditions during storage.

3. Conclusions

Results obtained indicate that the basic enzymes of glucidic metabolism are active in sucrose hydrolysis particularly in damaged beets stored in the interior of a pile. Moreover factory silos provide worst storage conditions than field piles. Even though the study of proteolytic activities is a useful tool for the investigation of the metabolism of beet roots, this is not an index of total amino acid amount present in factory raw juice.

*

This research was supported by a grant from Ministero della Publica Istruzione (40%).

Literature

Akeson, W. R. & Stout, E. L. (1978): Effect of impact damage on sucrose loss in sugarbeets during storage. *J. Am. Soc. Sug. Beet Technol., 20*, 167–173.

Baraldi, G., Bentini, M., Vaccari, G., Sgualdino, G. & Marzola, M. G. (1983): Danneggiamento della barbabietola nella fase di carico con benna. *Industria saccar. ital., 76*, 69–72.

Baraldi, G., Bentini, M., Spettoli, P., Vaccari, G., Marzola, M. G. & Sgualdino, G. (1984): Valutazione del danno da benna e conseguenti implicazioni chimiche ed enzimatiche sulla conservazione in cumulo di radici di bietola. *Industria saccar. ital., 77*, 47–54.

Bentini, M., Baraldi, G., Spettoli, P., Vaccari, G., Sgualdino, G. & Mantovani, G. (1987): Conservazione in cumulo di barbabietole da zucchero raccolte da terreni asciutti ed umidi. *Agricoltura mediterranea, 117*, 183–191.

Bradford, M. M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem., 72*, 248–254.

Burba, M. & Nitzschke, U. (1980): Nachweis und Eigenschaften der Saccharose spaltenden Enzyme der Zuckerrübe. *Zuckerindustrie, 105*, 149–153.

Cole, D. F. (1977): Effect of cultivar and mechanical damage on respiration and storability of sugarbeet roots. *J. Am. Soc. Sug. Beet Technol., 19*, 240–245.

Desmazeaud, M. J. & Juge, M. (1976): Caracterization de l'activité proteolytique et fractionnement des dipeptidase et des aminopeptidase de *Streptococcus thermophilus*. *Lait, 56*, 241–260.

Mc Cready, R. M. & Goodwin, J. C. (1966): Sugar transformation in stored sugar beets. *J. Am. Soc. Sug. Beet Technol., 14*, 197–205.

DEXTER, S. T., FRAKES, M. G. & GRANT, N. (1966): The effect of low, medium and high nitrogen fertilizer rates on the storage of sugarbeet roots at high and low temperatures. *J. Am. Soc. Sug. Beet Technol., 14,* 147–159.

PETERSON, C. L., TRAVELLER, D. J. & HALL, M. C. (1980): Loss of sucrose during controlled and conventional storage. *J. Am. Soc. Sug. Beet Technol., 20,* 517–530.

VACCARI, G., SPETTOLI, P., SGUALDINO, G. & MANTOVANI, G. (1983): Bietole danneggiate e tempo di sosta: Variazioni del valore tecnologico e di attivita enzimatiche del metabolismo glucidico. *Industria saccar. ital., 76,* 40–44.

VACCARI, G., MARZOLA, M. G., MANTOVANI, M., BENTINI, G., BARALDI, G. & SPETTOLI, P. (1988): Chemical and enzymatic changes in strongly damaged beets. *Fd Chem., 27,* 203–211.

VUKOV, K. & HANGYÁL, K. (1985): Sugar beet storage. *Sug. Technol. Rev., 12,* 143–265.

WYSE, R. (1974): Enzymes involved in the postharvest degradation of sucrose in *Beta vulgaris* L. root tissue. *Pl. Physiol., 53,* 507–508.

WYSE, R. E. (1978): Effect of injury on respiration and sucrose loss in sugarbeet roots during storage. *J. Am. Soc. Sug. Beet Technol., 20,* 193–202.

WYSE, R. E. & DEXTER, S. T. (1971): Effect of agronomic and storage practices on raffinose, reducing sugars and aminoacids content of sugar varieties. *J. Am. Soc. Sug. Beet Technol., 16,* 369–383.

WYSE, R. E. & PETERSON, C. L. (1979): Effect of injury on respiration rates of sugar beet roots. *J. Am. Soc. Sug. Beet Technol., 20,* 269–280.

Acta Alimentaria, Vol. 19 (1), pp. 63—72 (1990)

INFLUENCE OF BORON ON QUALITY ATTRIBUTES OF TOMATO FRUIT

J. A. ANKUSH,[a] L. HARGITAI,[a] P. A. BIACS[b] and H. G. DAOOD[b]

[a] Soil Science Department, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35—43. Hungary
[b] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 9 May 1989; accepted: 24 July 1989)

It is well known that under deficiency and toxicity conditions of an essential micronutrient such as boron (B), the quantity as well quality of plant products are substantially reduced. In this work, effect of B at different concentrations, alone or in combination with K, on the yield and quality attributes of tomato were investigated using an artificial soil of high organic N content "Vegasca". It was found that, without applied K, 6 ppm of B given to the plants, increased significantly the yield of tomato. Boron doses of 9 and 18 ppm resulted a remarkable decrease in the yield under the conditions used. The lower concentrations of applied B were found to improve quality attributes especially with application of K. But at higher dose (18 ppm), some quality attributes such as carotenoid, ascorbic acid, citric acid and sugar content, were detracted to high extent. High amount of B was more detrimental to the quality of tomato when K was applied.

Keywords: tomato quality, boron effect, HPLC, artificial soil

Boron is one of the elements essential for plant growth. Many evidences have been given to generally show that B was important in cell division and was apparently a necessary component of the cell wall (JACKSON & CHAPMAN, 1975; COHEN & LEPPER, 1977). The role of B in plant physiology, nutrition and growth has been reviewed in detail (GUPTA, 1979; 1985). The reviewed knowledge concerning the range between deficiency and toxicity of B indicated that the amount needed for normal crop growth and reproduction is different among various plants. At both deficiency and toxicity level the quality attributes of plant products are substantially influenced. Recently BELVER and co-workers (1987) confirmed that B has an action on ethylene production and lipoxygenase activity in microsomes from sunflower cotyledons. Such action may alternatively influence the dynamics of fruit ripening and the technological properties as well.

The purpose of this work was to study the effects of different doses of B on the quality characteristics of tomato fruits harvested from plants grown in Vegasca soil (rich in organic matter) with and without excessive supply of potassium. Also we aimed at the optimum dose of B for tomatoes with the best quality and quantity attributes.

1. Materials and methods

1.1. Plant material

Tomato seeds *(Lycopersion esculentum* cv. Balka) were germinated in Vegasca soil (from the Company of Soil Fertility, Sopron, Hungary). Upon reaching a height of 25–30 cm, the plants were transferred to pots containing 7 kg of Vegasca soil mixture and grown under plastic house conditions. Chemical composition of Vegasca soil used in this work is shown in Table 1. Treatments were started after 4 weeks of planting as shown in Table 2. The fruits were allowed to ripen on the plants. Eighteen fruits from each treatments (6 plants per treatment) were mixed and divided into three groups as replications. Two days before and after the treatments the plants were not watered.

1.2. Extraction methods

The fruits of each group were cut into small pieces with a plastic knife and samples of 25 g were disintegrated in a mortar and pestle with quartz sand.

Table 1

Chemical composition of Vegasca soil mixture

Constituents	Year of manufacturing		
	1985	1986	1987
Density (g cm^{-3})	0.80	0.77	0.75
Humidity	4.98	5.85	4.60
pH	6.75	6.40	6.50
$CaCO_3$ (%)	2.41	1.90	2.14
Humus (%)	27.04	30.40	29.30
Stability coefficient	0.046	0.032	0.086
T. S. S.	1.418	1.31	1.42
C/N	25.13	27.4	29.3
Available Ca (mg per 100 g)	298.63	270.30	296.3
Available Mg (mg per 100 g)	89.52	84.50	86.2
Total N (mg per 100 g)	1076	1170	950.3
Total P (mg per 100 g)	178.5	166.3	168.3
Total K (mg per 100 g)	860.4	760.4	930
Available N (mg per 100 g)	49.3	60.2	64.4
Available P_2O_5 (mg per 100 g)	153.10	160.2	162.4
Available K_2O (mg per 100 g)	258.19	260.40	270
NH_4-N (mg per 100 g)	140.5	149.2	155.2
NO_3-N (mg per 100 g)	245.5	250.2	247.3

Table 2

Addition of B[a] to Vegasca soil during growth of tomato plants

Weeks after planting	Dose of B (ppm)							
	with K[b]				without K			
4	0	3	3	6	0	3	3	6
8	0	3	6	12	0	3	6	12
Total	0	6	9	18	0	6	9	18

[a] Stock solution of borax were prepared and applied to the soils after suitable dilution

[b] K was applied at a concentration of 156 ppm

Pigment extraction was carried out by a method described in details by DAOOD and co-workers (1987). The method involved removal of water by methanol followed by pigment extraction with carbon tetrachloride–methanol 2 : 1. The CCl_4 layer was then dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuum in a rotary evaporator. The residues were kept under N_2 gas in refrigeration until HPLC analysis.

Soluble sugars and organic acids were extracted by a 4% metaphosphoric acid method (BIACS et al., 1987). After shaking for 30 min the homogenate was filtered and kept at -20 °C when not in use.

1.3. Chromatographic analysis

A Beckman series liquid chromatograph equipped with a Model 114 solvent delivery pump, a Model 165 variable wavelength detector, a Model 420 controller and a Model 142 injector, was used. The detector signals were electronically recorded on Shimadzu C-3RA integrator. The conditions and parameters of the different analyses were summarized in Table 3.

Table 3

HPLC conditions of different analyses for tomato constituents

Parameters	Conditions		
	Pigments	Organic acids	Sugars
Column	Chromsil C-18 10 μm, 46×250 mm	Lichosorb C-18 10 μm, 4.6×250 mm	Chromsil–H_2 10 μm, 4.6×250 mm
Eluent	Acetone–water (90 : 10)	0.1 M KH_2PO_4 (950 cm^3), methanol (30 cm^3) Tetrabutyl ammonium hydroxide (0.5 cm^3)	Acetonitrile–water (80 : 20)
Flow rate	1 cm^3 min^{-1}	1 cm^3 min^{-1}	1 cm^3 min^{-1}
Detection	438 nm	225 nm	R. I.×8

1.4. Identification of components

Sugars and organic acids were identified according to the retention times on the HPLC chromatograms and compared with those of the standards (Sigma, USA). Internal standards were also used for further identification.

Carotenoid pigments were identified as previously described (DAOOD et al., 1987). Identification of carotenoid type pigments required, in addition to the use of authentic standards, scanning the maximum absorption wavelength of each component and comparing them with the values reported in the literature (CURLE, 1961; BICKLE & RAHMAN, 1979). Quantification of β-carotene was achieved by using pure standard pigments (Sigma) whereas, peak areas were used to represent the amount of other pigments the pure standard of which was not available.

2. Results and discussion

2.1. Yield of tomato

It is a well-known fact that both deficiency and excess of any essential element will result in reduction of crop yield and/or in impairment of crop quality. Figure 1 shows that treatment with 6 ppm B without K significantly increased tomato yield, but further addition (9 and 18 ppm) increased the yield only slightly. On the other hand, linear decline in tomato yield was

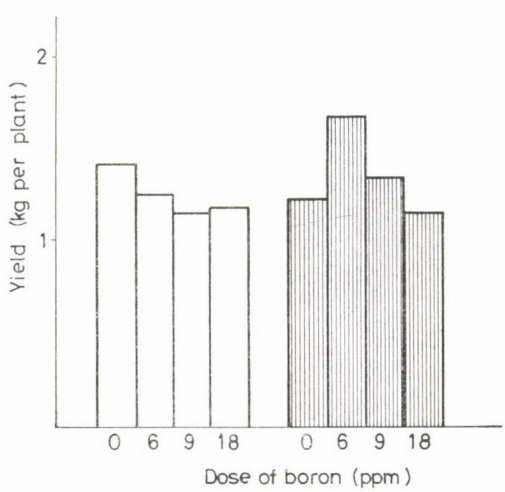


Fig. 1. Effect of boron treatment on the production of tomato plants grown in Vegasca soil mixture. The values represent the averages of two years (1986, 1987).
□: With K; ▨: without K

recorded with the increase of B dose. Such results suggest that excess of K decrease the yield when the plants are under stress of B. It could also be concluded that yield increasing action of K can be replaced by enrichment of soil with B within the sufficiency level. These results reveal the effective interaction between B and K both in the soil and in the growing plant as well.

2.2. Carotenoid content

Figure 2 shows the HPLC chromatogram of tomato extract on reversed--phase Chromsil C-18 column. In addition to the major pigments (lycopene and carotene) oxygen containing xanthophylls such as lutein and lycoxanthin could also be detected.

Boron was found to increase lycopene content of tomato fruit when added to the soil. The increase was proportional to the increasing rate of B at both early and final stages of ripeness (Fig. 3), particularly when K was not applied in excess. In the case of K application, tomato fruit harvested from plants grown under B toxicity conditions (18 ppm) contained less lycopene

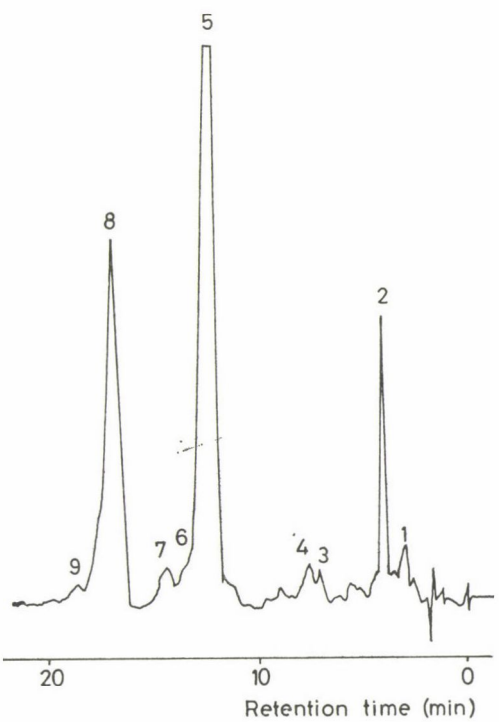


Fig. 2. HPLC profile of tomato fruit pigments separated on Chromsil C-18 column and eluted with acetone—water (90 : 10) as the mobile phase. 1: Neoxanthin; 2: lutein; 3: lycoxanthin b; 4: lycoxanthin a; 5: lycopene; 6: neurosporene; 7: γ-carotene; 8: β-carotene; 9: ζ-carotene

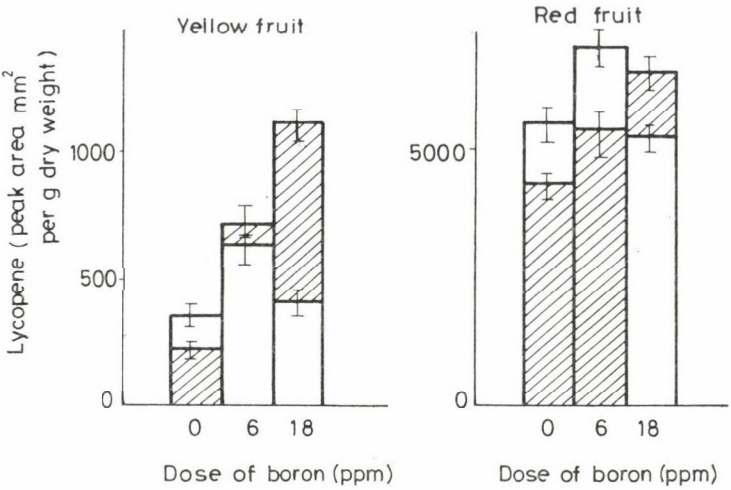


Fig. 3. Changes in lycopene content of mature yellow and mature red tomato fruit as a result of boron treatments. □: With K; ▨: without K; I: standard deviation

in comparison with those obtained from other treatments. This may be due to the enhancement of lycopene to xanthophyll transformation as a result of B toxicity. Similar tendency was observed at the final stage of ripeness. When B was not added (control) application of K caused an increase in the lycopene content of tomato fruit confirming the results obtained by TRUDEL and OZBUN (1971).

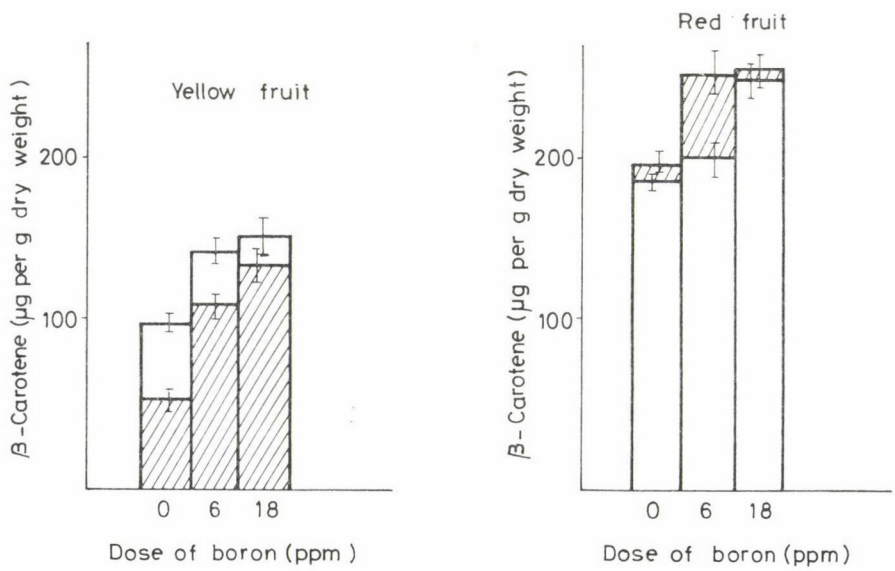


Fig. 4. Effect of boron treatments on the β-carotene content of tomato fruit harvested at different stages of ripeness. □: With K; ▨: without K; I: standard deviation

As for β-carotene, increasing amount of B increased provitamin A content of tomato harvested from both K-treated and untreated plants (Fig. 4). At a low concentration of B (particularly at 6 ppm) K favourably influenced β-carotene synthesis but no significant difference was observed at B in excess. It could also be observed that plants treated with K yielded yellow fruits of relatively high β-carotene content. Such results revealed that B may promote cyclization of lycopene to β-carotene at the early stage of ripening in the presence of high amount of K in the soil. A special attention should be paid to these results particularly when vitamin A precursors form the aim of the research work.

2.3. Organic acid content

Figure 5 shows HPLC profile of tomato extract on ion-pair Lichosorb C-18 column. Several acids could be separated and identified from tomato extract. In this paper we dealt with only three of them definitely: ascorbic, citric and malic acid, the main characteristic acids of tomato.

In the case of K addition, ascorbic acid reached maximum value of 6 ppm of B. As B dose further increased ascorbic acid content of the fruit decreased (Fig. 6). The same tendency was observed when K was not added. The positive cooperation between K and B can be seen when 6 ppm of B did

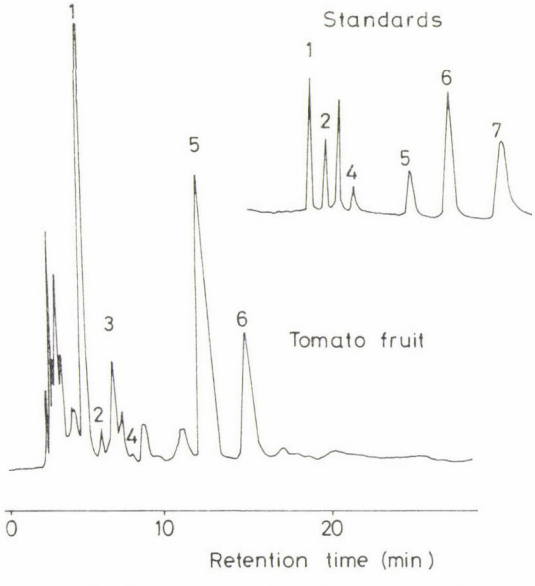


Fig. 5. HPLC profile of standard organic acids and tomato fruit extract on Lichosorb C-18 column under ion-pair chromatographic conditions. 1: Ascorbic acid; 2: malic acid; 3: tartaric acid; 4: succinic acid; 5: citric acid; 6: fumaric acid; 7: gallic acid

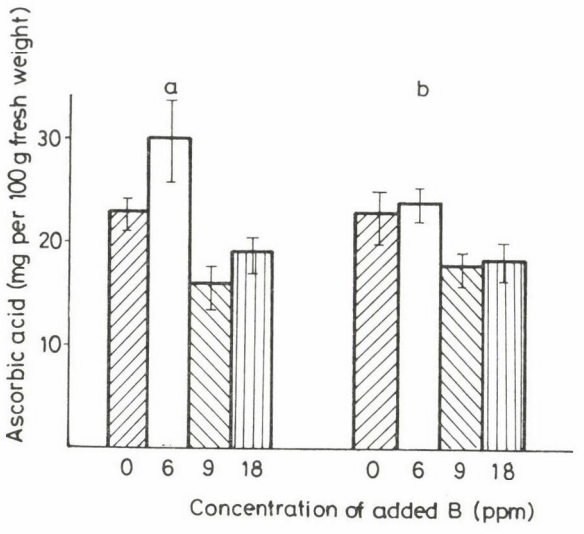


Fig. 6. Effect of boron treatments on ascorbic acid content of tomato fruit. The values are the means of 3–4 replications. a: With K; b: without K; I: standard deviation

not significantly increase ascorbic acid content in the case of K-untreated samples. In general, excess of B exerted a disturbing effect on ascorbic acid synthesis or promoted its oxidative damage during the ripening process.

Citric acid generally increased with increasing dose of B both with and without K addition. At B dose of 0 ppm addition of K significantly increased citric acid content (Fig. 7) indicating the activating effect of K on citric acid

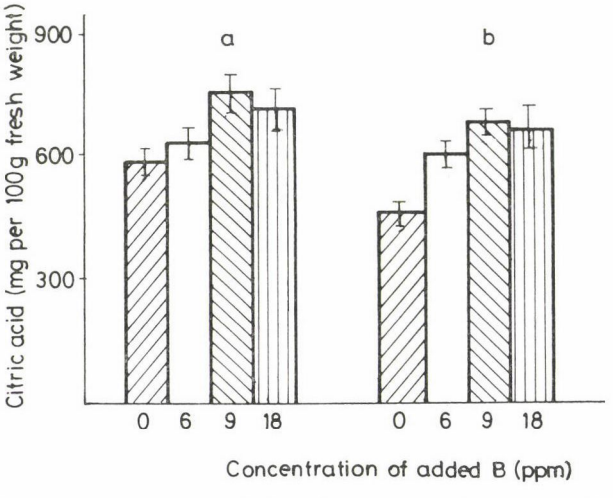


Fig. 7. Effect of boron treatments on citric acid content of tomato fruit. The values are the means of 3–4 replications. a: With K; b: without K; I: standard deviation

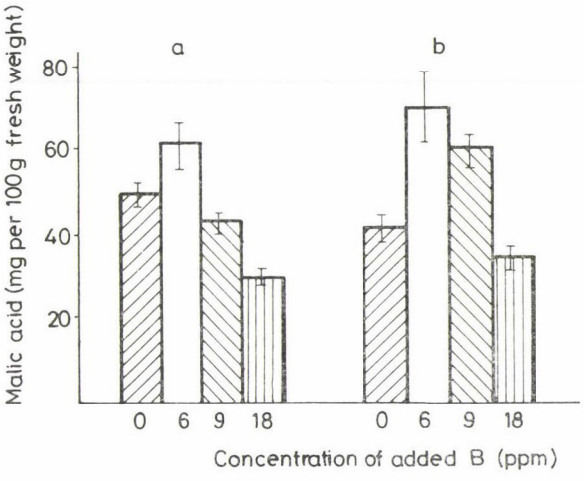


Fig. 8. Effect of boron treatments on malic acid content of tomato fruit (means of 3–4 replications). a: With K; b: without K; I: standard deviation

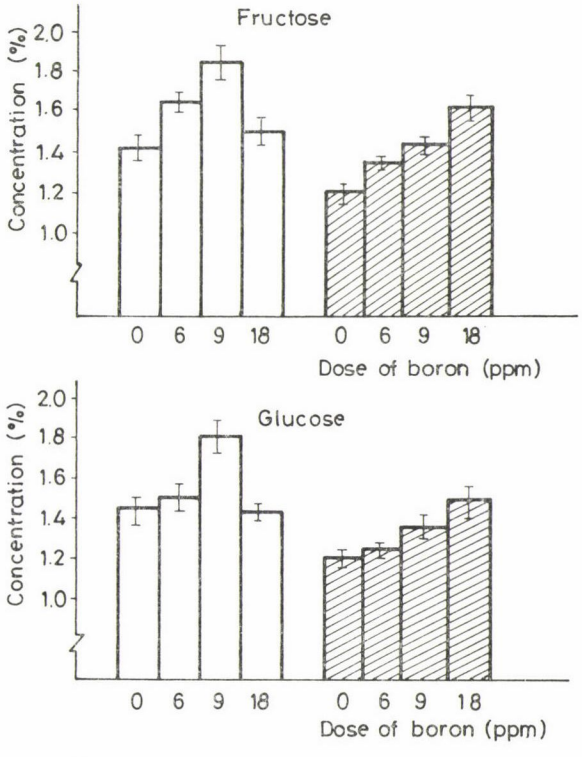


Fig. 9. Effect of boron dose on reducing sugar concentration in tomato fruit. The results represent average of three replications. □: With K; ▨: without K; I: standard deviation

synthesis. Unlike citric acid, malic acid decreased as B dose increased (Fig. 8) revealing the inverse correlation between citric and malic acid. Such correlation has been observed in many vegetables such as sweet potato (PICHA, 1985) and tomatoes (DAVIES & HOBSON, 1981).

2.4. Sugar content

Since K is a well-known macro-element having important role in synthesis and translocation of sugars in plants, its addition to the soil increased fructose and glucose content in tomatoes (Fig. 9). Boron dose, when increased, caused linear increase in sugar concentration except at treatment with B of 18 ppm when the sugar content declined. Low sugar content of tomato fruit harvested from plants grown in soil highly fortified with B and K may relate to the toxicity symptoms which influence to a higher extent the photosynthetic process, especially carbohydrate synthesis in plant leaves. It is important to mention that plants treated with a high dose of B and K showed toxicity symptoms on their leaves.

Literature

BIACS, P., DAOOD, H., PAIS, I., FEHÉR, M. & HAJDU, F. (1987): Chromatographic investigation of tomato fruit metabolites during ripening. – in: KALÁSZ, H. & ETTRE, L. (Eds.) *Chromatography '87*. Akadémiai Kiadó, Budapest, pp. 39–59.

BELVER, A., RODRIGUEZ, P., ROLDAN, M. & DONAIRE, J. P. (1987): Action of boron on ethylene production and lipoxygenase activity in microsomes from sunflower cotyledons. – in: STUMPF, P. K., MUDD, J. B. & NES, W. D. (Eds) *The metabolism, structure and function of plant lipids*. Plenum, New York, pp. 417–419.

BICKLE, K. A. & RAHMAN, M. M. (1979): Separation of chlorophyll and carotenoid pigments of capsicum cultivars. *J. Chromat., 171*, 385–391.

COHEN, M. S. & LEPPER, R., Jr. (1977): Effect of boron on cell elongation and division in squash root. *Pl. Physiol., 59*, 884–887.

CURLE, A. L. (1961): The xanthophylls of tomato. *J. Fd Sci., 26*, 106–111.

DAOOD, H., BIACS, P., HOSCHKE, Á., HARKAY-VINKLER, M. & HAJDU, F. (1987): Separation and identification of tomato fruit pigments by TLC and HPLC. *Acta Alimentaria, 16*, 339–350.

DAVIES, J. N. & HOBSON, G. E. (1981): The constituents of tomato fruit, the influence of environment, nutrition and genotype. *CRC Crit. Rev. Fd Sci. Nutr., 15*, 204–230.

GUPTA, U. C. (1979): Boron nutrition of crops. *Adv. Agron., 31*, 273–307.

GUPTA, U. C. (1985): Boron toxicity and deficiency: A review. *Can. J. Soil. Sci., 65*, 381–409.

JACKSON, J. F. & CHAPMAN, K. S. R. (1975): The role of boron in plants. – in: NICHOLAS, D. J. D. & EGAN, A. R. (Eds.) *Trace elements in soil – plant – animal system*. Academic Press, New York, pp. 213–225.

PICHA, D. H. (1985): Organic acid determination in sweet potatoes by HPLC. *J. agric. Fd Chem., 33*, 343–745.

TRUEL, M. J. & OZBUN, J. L. (1971): Influence of potassium on carotenoid content of tomato fruit. *J. Am. Soc. hort. Sci., 96*, 763–765.

Acta Alimentaria, Vol. 19 (1), pp. 73—78 (1990)

CHARACTERIZATION OF PEPTIDES ENRICHED IN METHIONINE BY ENZYMATIC PEPTIDE MODIFICATION

GY. HAJÓS,[a] H. NÖTZOLD,[b] A. HALÁSZ[a] and E. LUDWIG[b]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] Department of Chemistry, Technical University of Dresden, 8027 Dresden, Mommsenstrasse 13. GDR

(Received: 16 June 1989; accepted: 7 July 1989)

Methionine-enriched polypeptides were produced from an enzymatically prehydrolyzed milk protein and L-methionine ethyl ester hydrochloride by application of enzymatic peptide modification (EPM) method using α-chymotrypsin as catalyst. Methionine content of the product was more than twice as high as that of the substrate. The peptides of the EPM-product and the substrate were separated by thin-layer chromatography, and were then subjected to amino acid determination.

The ratio of the polar and apolar amino acids of the peptides was found to be influenced basically by transpeptidation taking place in the EPM-reaction.

Analysis of the peptides of these products also showed that methionine was incorporated mainly in those peptides containing a relatively high ratio of apolar amino acids.

Keywords: separation of peptide mixtures, enzymatic peptide modification, characterization of peptides, methionine-enriched polypeptides

The enzymatic peptide modification (EPM) (HAJÓS, 1986), a procedure involving enzymatic catalysis, proved to be a suitable method to produce polypeptides of designed amino acid composition (YAMASHITA et al., 1976; FRUTON, 1982; ASHLEY et al., 1983; ASO et al., 1985; HAJÓS et al., 1988a, b).

In the literature there is still much debate about the actual reaction mechanism (YAMASHITA et al., 1973; EDWARDS & SHIPE, 1978; NOAR & SHIPE, 1984; LUDWIG et al., 1981; GAERTNER et al., 1982), and thereby also about the hydrophobic character of the reaction products of the plastein reaction, and of the enzymatic peptide modification.

ANDREWS (1985) reviewed that plasteins were formed by an association of predominantly hydrophobic peptides via hydrophobic and possibly ionic bonding.

ASO and his co-workers (1974) concluded that hydrophobic forces are a major factor in plastein chain assembly. They found that compared with the substrate, the water-insoluble product contained smaller amounts of

Offprint requests to: Gy. Hajós

hydrophilic and larger amounts of hydrophobic amino acid residues. The results of SUKAN and ANDREWS (1982) showed that hydrophobic amino acids such as phenylalanine, leucine, isoleucine, tyrosine, valine and proline were preferentially incorporated into plastein at the expense of hydrophilic amino acids.

In some other papers a trend of a prefential incorporation of hydrophobic amino acids into the protein product in the enzyme-catalysed reactions has been reported, too (HOROWITZ & HAUROWITZ, 1959; ERIKSEN & FAGERSON, 1976; FUJIMAKI et al., 1977; NÖTZOLD et al., 1983; WINKLER et al., 1988).

Although certain hydrophobic amino acids were found to concentrate in some EPM samples in exceptional cases, investigation in this respect carried out with Purina E-500 and casein did not provide any evidence for generalization of this finding (HAJÓS, 1988).

Our earlier studies revealed that transpeptidation is the major process in the enzymatic peptide modification (HAJÓS et al., 1988b) and that the presence of a great amount of amino acid derivatives in the reaction mixture significantly modifies the process of the transpeptidation.

The aim of the present study was to determine the influence of transpeptidation on the ratio of the polar and apolar amino acids of the peptides in the reaction mixture, and the tendency of the methionine incorporation in respect of the hydrophobic character of the peptide chains.

1. Materials and methods

An enzymatically prehydrolyzed commercial milk protein concentrate, Sportrobi (SR) was purchased from Répcelaki Sajtgyár (Hungary). The α-Chymotrypsin, EC 3.4.21.1 (Sigma) was used. L-methionine was esterified with ethanol and dry hydrogen chloride and the reaction product was crystallized from a mixture of ethanol and diethylether to give L-methionine ethyl ester hydrochloride (M.p. 90–91 °C).

1.1. Enzymatic peptide modification (EPM)

The enzymatically prehydrolyzed milk protein (SR) was used as substrate without further hydrolysis. The concentration of the substrate was 25% w/v. The ratio of methionine ethyl ester hydrochloride–substrate was 1 : 5, and α-chymotrypsin was used as catalyst. The enzyme–protein ratio was 1 : 100. The incubation was carried out at 37 °C (pH: 6.0).

The substrate and the EPM product were, after incubation, simultaneously dialyzed for 48 h through a cellophane membrane against distilled water. The nondialyzable fraction was freeze-dried.

1.2. Determination of amino acid composition

An aliquot of the samples was hydrolyzed with 6 mol l^{-1} HCl in a tube flushed with N_2 at 110 °C for 24 h. The samples hydrolyzed with HCl were analyzed for amino acids by an amino acid analyzer (AAA339T, Mikrotechna, Praha).

1.3. Determination of methionine by microbiological methods

The methionine content was determined from the hydrochloric acid hydrolysates of the samples according to BARTON-WRIGHT (1977) by using *Leuconostoc mesenteroides* P60 auxotroph mutant, as a test organism.

1.4. Separation by thin-layer chromatography

Merck DC Kieselgel 60 (with concentrating zone) plates were used. Running system phenol–water 75 : 25 (w/w), running distance 10 cm, running time 3 h.

The R_F values were determined from 20 parallel separations. Three mg of the samples were dissolved in 1 cm^3 of solvent and 20 μl was applied to cover a distance of 2 cm in the concentrating zone.

2. Results and discussion

Methionine-enriched protein was produced from SR by the EPM process, and the methionine contents of SR and the EPM product were determined by microbiological method (Table 1). Methionine of the EPM product was more than twice as high as that of the substrate.

Table 1

Methionine content of SR and of the methionine-enriched EPM product

Samples	Methionine[a] (%)
(A): SR[b]	3.13
(B): EPM product from SR with methionine enrichment	6.47

[a] Methionine of the samples is given as per cent of the total amino acids, determined by microbiological methods

[b] Enzymatically prehydrolyzed commercial milk protein concentrate

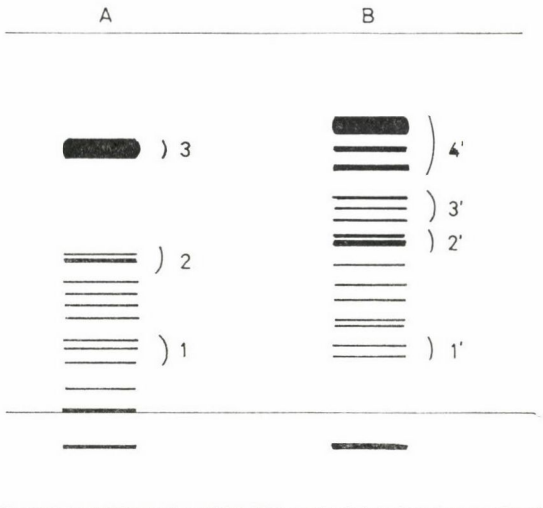


Fig. 1. Separation of protein fractions on thin-layer chromatography. A: Sportrobi, an enzymatically prehydrolyzed milk protein (SR); B: EPM product with methionine enrichment (prepared from Sportrobi by enzymatic peptide modification using L-Met ethyl ester, with α-chymotrypsin catalysis). Thin-layer chromatography was carried out on Merck DC Kieselgel 60 (with concentrating zone) plate.

Separation of peptides of the EPM-product and substrate containing the polar and apolar amino acids in different amounts, was carried out on Kieselgel.

The chromatograms of the substrate (A) and of the EPM product (B) as shown in Fig. 1, are significantly different. More than the half of the peptide zones of the methionine-enriched product showed a different mobility on the thin-layer.

The R_F values of peptide fractions of SR and EPM products were determined on the basis of 20 parallel separations and were found as follows:

Sportrobi (sample A)

0.06, 0.13, 0.17, 0.19, 0.25, 0.28, 0.31, 0.34, 0.40, 0.41 and 0.70.

EPM product (sample B)

0.15, 0.18, 0.23, 0.25, 0.30, 0.34, 0.39, 0.45, 0.46, 0.51, 0.54, 0.57, 0.65, 0.70 and 0.76.

The separated peptides and peptid mixtures were eluted and their molar amino acid content was determined. The amino acid content of a few selected peptide fractions is summarized in Table 2. The results of the amino acid analysis suggested that the separation proceeded primarily according to the ratio of the polar and apolar amino acids of the peptide fractions.

Table 2

The molar (%) amino acid content[a] of peptide fraction by thin-layer chromatography

	A			B			
	1	2	3	1′	2′	3′	4′
Lys	4.6	3.2	0.8	4.3	3.1	3.7	3.0
His	5.9	4.2	5.3	5.5	5.2	5.7	4.0
Arg	3.6	0.1	0.1	3.7	2.5	1.1	1.5
Asn	9.1	6.0	5.2	7.8	9.1	7.0	6.5
Thr	5.8	2.7	2.7	4.0	5.3	6.3	3.9
Ser	8.0	6.8	6.5	6.0	6.1	5.8	4.8
Glu	19.3	6.2	4.3	14.5	9.7	9.3	6.6
Pro	5.7	4.7	5.2	6.0	6.2	5.0	4.7
Gly	15.1	22.4	25.4	20.2	15.4	9.7	19.6
Ala	4.5	9.4	9.2	11.1	8.3	6.0	9.5
Cys[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Val	3.5	5.2	6.4	4.0	5.0	6.8	5.5
Met[b]	1.2	1.0	1.1	0.3	0.8	3.0	5.8
Ile	3.4	6.2	6.4	3.6	3.5	4.8	3.1
Leu	5.2	9.4	6.7	5.0	9.7	13.8	10.4
Tyr	2.3	8.7	10.5	2.1	6.3	6.9	5.7
Phe	2.7	3.8	4.2	1.8	3.7	5.0	5.3

A: SR (Sportrobi), enzymatic prehydrolyzed milk protein
B: EPM product, produced from SR in the presence of α-chymotrypsin and L-Met ethyl ester by enzymatic peptide modification
The numeration of peptides and peptide-fractions can be seen in Fig. 1

[a] The error of measurements is cca 10%
[b] Half-cystine and methionine were determined separately as cysteic acid and methionine sulfone, respectively, after performic acid procedure

It is remarkable, that the molar ratio of Asn, Thr, Ser and Glu decreases with increasing R_F values of the peptides. The molar ratio of Phe, however, keeps growing with the increase of R_F values of the peptide zones.

The molar ratio of the apolar amino acids, e.g. Val and Leu, shows a significant increase, especially between the zones 1 and 2, and 1′ and 2′.

The number of the peptide zones in the range of R_F values of 0.40 and 0.76 of the EPM product increased strikingly. Further refinement of both the high R_F zones (0.70 and 0.76) and zones with small ratio of polar amino acids (0.15 and 0.18) seems to be beyond the limit of the technique used.

Since methionine was found to concentrate mainly in peptide zones 3′ and 4′ (see Fig. 1 and Table 2), we can conclude that under these conditions,

the methionine did not incorporate in peptides containing high ratio of polar amino acids.

These chromatographic separations based on differentiation by polarity of the components provided a novel approach for characterization of the separated peptides.

Literature

ANDREWS, T. A. (1985): Proteinases and their effect on milk and other food proteins (Review). Food Research Institute Reading; Report, April 1985–86.

ASHLEY, D. V. M., TEMPLER, R., BARCLAY, D., DORMOND, C. A. & JOST, R. (1983): Amino acid-enriched plasteins: a source of limiting amino acids for the Weanling rat. *J. Nutr., 113,* 21–27.

ASO, K., KIMURA, H., WATANABE, M. & ARAI, S. (1985): Chemical properties of enzymatically modified proteins produced from soy protein by covalent attachment of methionine. *Agric. biol. Chem., 49,* 1649–1654.

ASO, K., YAMASHITA, M., ARAI, S. & FUJIMAKI, M. (1974): Tryptophan-, threonin- and lysine-enriched plasteins from zein. *Agric. biol. Chem., 38,* 679–680.

BARTON-WRIGHT, E. (1977): The microbial assay of the essential amino acids in compound feeding stuffs. *Analyst, 97,* 138–141.

EDWARDS, J. H. & SHIPE, W. F. (1978): Characterization of plastein reaction products formed by pepsin, α-chymotrypsin, and papain treatment of egg albumin hydrolysates. *J. Fd Sci., 43,* 1215–1218.

ERIKSEN, S. & FAGERSON, I. S. (1976): The plastein reaction and its applications: A review. *J. Fd Sci., 41,* 490–493.

FRUTON, J. S. (1982): Proteinase-catalyzed synthesis of peptide bonds. *Adv. Enzymol., 53.* 239–306.

FUJIMAKI, M., ARAI, S. & YAMASHITA, M. (1977): Enzymatic protein degradation and resynthesis for protein improvement. *Adv. Chem. Ser., 160,* 156–184.

GAERTNER, H., LUPI, N. & PUIGSERVER, A. (1982): Greffage covalent d'aminoacides essentiels sur les protéines alimentaires. *Lait, 62,* 578–599.

HAJÓS, GY. (1986): Incorporation of essential amino acids into protein by enzymatic peptide modification (EPM). *Nahrung, 30,* 418–419.

HAJÓS, GY. (1988): *Élelmiszerfehérjék enzimes módosítása.* (Enzymatic modification of food proteins.) Thesis, Budapest, 136 pp.

HAJÓS, GY., ÉLIÁS, I. & HALÁSZ, A. (1988a): Methionine enrichment of milk protein by enzymatic peptide modification. *J. Fd Sci., 53,* 739–742.

HAJÓS, GY., MIETSCH, F. & HALÁSZ, A. (1988b): Change of degree of hydrolysis in the course of enzymatic peptide modification. *Acta Alimentaria, 17,* 265–270.

HOROWITZ, J. & HAUROWITZ, F. (1959): Mechanism of plastein formation. *Biochem. biophys. Acta, 33,* 231–238.

LUDWIG, E., KRAUSE, W., NÖTZOLD, H. & TEBLING, F. (1981): Enzymatische Modifizierung (Plasteinsynthese) von Ackerbohnen-Protein. *Nahrung, 25,* K15–K17.

NOAR, S. R. & SHIPE, W. F. (1984): A study of the forces involved in the incorporation of L-methionine into soy protein by the one-step plastein-like process. *J. Fd Sci., 49,* 1316–1318.

NÖTZOLD, H., SCHÄFER, H. & LUDWIG, E. (1983): Zur potentiometrischen Titration von Ackerbohnenproteinisolat und daraus hergestelltem Plastein. *Nahrung, 27,* 71–78.

SUKAN, G. & ANDREWS, A. T. (1982): Application of the plastein reaction to caseins and to skim-milk powder. *J. Dairy Res., 49,* 265–278.

WINKLER, H., NÖTZOLD, H. & LUDWIG, E. (1988): Plasteinbildung Anreicherung hydrophober Aminosäuren und Methionon-Einbau in peptische Partialhydrolysate des Ackerbohnenproteinisolats bei der durch Thermitase katalysierten Plasteinreaktion. *Nahrung, 32,* 135–143.

YAMASHITA, M., ARAI, S. & FUJIMAKI, M. (1976): Plastein reaction for food protein improvement. *J. agric. Fd Chem., 24,* 1100–1103.

YAMASHITA, M., ARAI, S., TANIMOTO, S. & FUJIMAKI, M. (1973): Condensation reaction occurring during plastein formation by α-chymotrypsin. *Agr. biol. Chem., 37,* 953.

Acta Alimentaria, Vol. 19 (1), pp. 79–86 (1990)

THE EFFECT OF PAPRIKA SEED ON THE STABILITY OF THE RED COLOUR OF GROUND PAPRIKA

M. Okos, T. Csorba and J. Szabad

Paprika Processing Enterprise, H-6701 Szeged, Szövetkezeti út 1. Hungary

(Received: 24 May 1988; accepted: 30 December 1988)

The loss of red colour in dried and ground paprika was controlled in various degrees by the addition of seeds of paprika. It is well known that the seed of paprika contains different types of fatty acids. These fatty acids can promote the discoloration of ground red pepper.

In the present paper this factor was investigated, using various quantities of paprika seed, in an attempt to find out whether this factor may be significant in causing discoloration. The addition of different percentages of ground paprika seed to ground paprika pericarp did not increase colour loss.

Percentage of the loss of colour did not differ after 5 months of storage, but at the end of the subsequent seven months the paprika containing seeds lost its colour to a lower degree than the paprika without seeds.

Keywords: red pepper seeds, changing of total pigment

Ground red pepper loses a part of its pigments during storage. In this part of our country cultivar Sz-20 is grown. Sometimes it occurs, that the final product of this variety loses its colour within six months. This is a very serious economic problem for our factory. Certainly there are a lot of reasons for this loss of colour. According to Lease and Lease (1956) the stability of the colour of the condiment depends to a high degree on the variety of the pepper grown. A good colour-stable variety is able to retain 70% of its initial colour stored in bottles during 12 months at 25 °C. The intensity of the initial colour, the percentage of moisture or of crude fat is not correlated with subsequent colour stability in a colour-stable variety. Light and air have an effect on the stability of colour in a colour-stable variety, too. A more colour-stable ground paprika remained bright on the inner surface for many months in glass bottles and even under free access of air. Lease and Lease (1962) investigated the effect of the drying conditions of paprika pods. They established, that sliced pods are preferred over hole pods for drying because of the shorter drying time required. A drying temperature of 65 °C appeared to combine good colour and rapid drying.

The moisture content of ground red paprika and the stage of ripeness of the fruits have also an effect on the colour deterioration of final product during

storage. Increasing the moisture content to 14% enhanced colour stability and precluded pigment destruction in ground red paprika.

The right stage of ripeness to get a good product is when the fruits are fully ripe but still succulent (KANNER et al., 1977).

Care must be taken to gather only the pods which are free from disease and other defects. It is very difficult in practice to choose the healthy pods. Spoiled pods can also be the reason for the discoloration of ground red paprika (NAGY & MURÁNYI-FEKETE SZÜCS, 1978).

Practical experience showed that (OM) the bright initial colour of ground red paprika is ascribed to ground seeds, therefore the seeds are ground together with the pericarp. But in the seeds there are fatty acids which can accelerate discoloration of ground red paprika.

In the present experiments we wanted to clarify the effect of the seeds.

1. Materials and methods

1.1. Materials

The material used in the experiments was the Hungarian cultivar Sz-20 obtained from our factory. All the solvents and chemical reagents used were of analytical grade, and they were obtained from Reanal, Hungary. The following solvents and chemical agents were applied: aceton, petroleum ether (b.p. 70 °C), anhydrous sodium sulfate, ether, benzene, glacial acetic acid, methanol, ethanol, chloroform, isopropanol, potassium hydroxide, DC-Alufolien Kieselgel 60 20 × 20 cm, layer thickness 0.2 mm, activated for 4 h at 130 °C.

1.2. Methods

1.2.1. Preparation of ground paprika. After removal of the stems and seed-vessels the red succulent pericarp of the paprika fruit was dried at 25 °C room temperature. The moisture content of the dried pericarp was about 10%. After drying the pericarp was ground, and passed through a sieve of 0.5 mm mesh.

The seeds were also ground, and 10–60% was added to the ground pericarp and thoroughly blended in a mortar.

1.2.2. Extraction. 0.1 g of the homogenized samples was extracted in 100 cm^3 aceton during 2 h.

Extraction was carried out in the dark, under continuous shaking. Then, the colour was measured at 460 nm wavelength, and the total pigment content was calculated according to HUNGARIAN STANDARD (1977) and SZABÓ and KOSA (1985).

1.2.3. Isolation of the oleoresin. Two to 5 g of the homogenized samples was consecutively treated with ether until a colourless drop was obtained. The joined extracts were evaporated to dryness in a rotary vacuum evaporator, and the residual oleoresin was weighted. The ground seed was treated in the same manner for 16 h.

1.2.4. Thin layer chromatography. Samples of 0.2 g were extracted with chloroform–aceton–isopropanol 2 : 1 : 1 mixture according to PAVISA and co-workers (1987). The extract was evaporated to dryness in a rotary vacuum evaporator. The dry residue was saponified with 10% KOH–ethanol overnight at room temperature. The carotenoids saponified were quantitatively transferred into diethyl ether. The solution was rinsed with distilled water to neutral reaction, then dehydrated over (OM) anhydrous Na_2SO_4, and evaporated to dryness under vacuum. The pigment adhering to the wall of the vessel was dissolved in aceton, then it was applied to the chromatography layer. The thin-layer chromatography and the quantities of capsanthin and capsorubin were determined according to VINKLER and KISZEL-RICHTER (1972).

2. Results and discussion

The samples were stored in plastic bags and the small plastic bags were placed in a bigger paper bag. The samples were kept in the dark, and were protected from oxygen of the air. The storage temperature changed between 12 and 25 °C. The humidity of air was not measured. The moisture content of the samples changed from 7 to 9% during storage. The initial colour was found to be the highest in samples obtained from pericarp only.

In other samples the initial colour content depended on the proportion of added seeds. But the difference between the initial and the final pigment content in the sample was the lowest at 60% seed content.

It can be seen that all the samples retained about 70 to 80% of their initial pigment content after nine months storage. In the eleventh month of storage a little difference can be seen among the samples as regards their pigment content. This trend becomes more apparent in the 12th month of storage. The pure pericarp lost 40% of its initial pigment content, while the sample containing 60% seed only 20%.

In this experiment the effect of added seeds on the stability of the pigment content of the ground red paprika became apparent during storage. Since there are lots of factors which affect the discoloration of ground red paprika, we used in the experiment selected and totally healthy paprika pods, and the paprika was ground in the laboratory. We tried to preclude those factors which affect the stability of colour during harvesting and grinding the paprika, in order to be able to establish the effect of ground seeds.

Table 1

Loss of colour in the samples of different seed content during storage

Time of storage (month)	Total pigment content (g per kg) seed per pericarp (%)													
	0		10		20		30		40		50		60	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
0	10.46	0.09	9.28	0.10	8.30	0.08	7.20	0.07	6.10	0.04	5.40	0.04	4.30	0.03
1	10.42	0.06	8.70	0.01	7.58	0.02	6.99	0.01	5.91	0.06	4.86	0.02	3.97	0.05
2	9.45	0.02	8.58	0.03	7.51	0.06	6.60	0.08	5.77	0.04	4.80	0.01	3.96	0.02
3	9.37	0.07	8.50	0.03	7.67	0.08	6.50	0.03	5.55	0.03	4.57	0.01	3.94	0.02
4	8.97	0.02	8.24	0.07	7.40	0.03	6.40	0.01	5.34	0.08	4.46	0.01	3.91	0.03
5	8.59	0.02	7.82	0.02	6.97	0.02	6.02	0.02	5.35	0.03	4.53	0.03	3.75	0.03
7	8.06	0.03	7.26	0.03	6.45	0.03	5.64	0.02	5.18	0.02	4.52	0.02	3.72	0.02
9	7.34	0.04	6.85	0.02	5.99	0.02	5.22	0.02	4.77	0.02	4.29	0.05	3.48	0.02
11	7.03	0.02	6.44	0.03	5.68	0.03	5.07	0.04	4.67	0.03	4.21	0.01	3.56	0.01
12	6.32	0.06	6.11	0.04	5.42	0.02	4.76	0.02	4.53	0.01	4.19	0.03	3.44	0.01
Oleoresin (%)	8	0.252	10	0.153	12	0.179	14	0.166	16	0.299	18	0.210	18	0.210

In percentage of dry material contents

x: mean value of three measurements; ±s: standard deviation

The crude fat content of the samples was increased from 8% to 18% by the addition of ground seed (Table 1). The seed contains high amounts of linoleic acid and linolenic acid, both susceptible to autoxidation.

The presence of these fatty acids, though common in paprika pod fats, can decrease the stability of carotenoids in paprika. Our results have shown that increasing the crude fat content by adding ground seeds to the ground

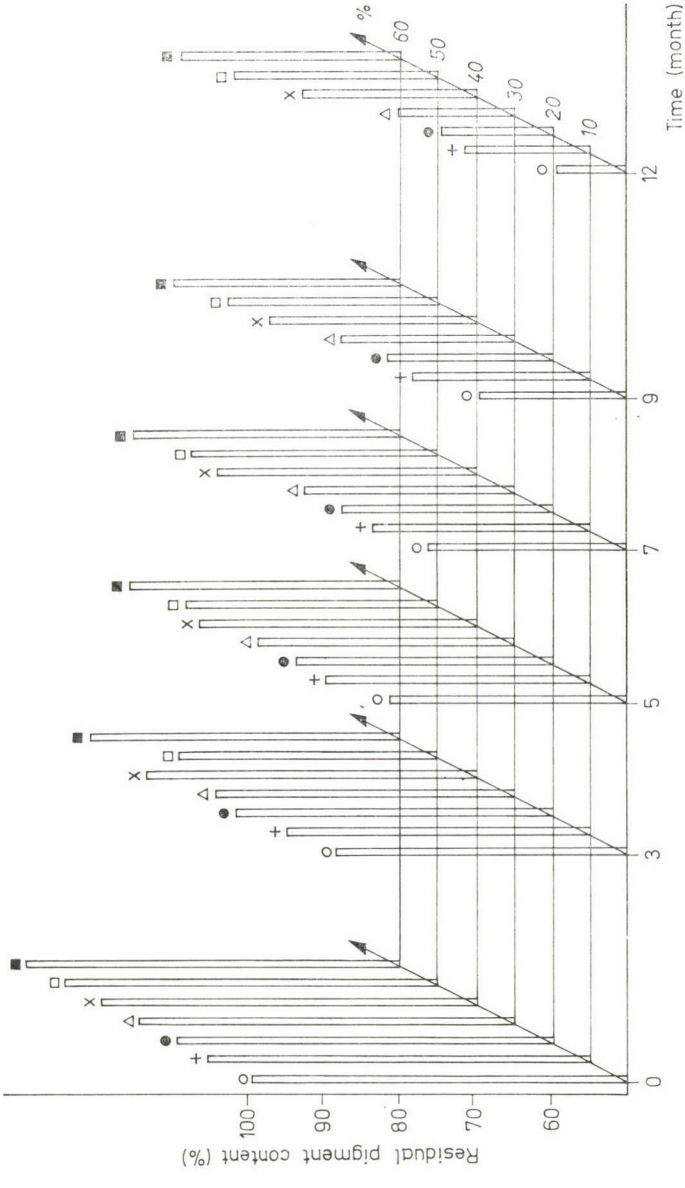


Fig. 1. The residual percentage of the initial pigment content in the samples of different, seed content, during storage. o: pure pericarp, +: 10% seed, ●: 20% seed, △: 30% seed, x: 40% seed, □: 50% seed, ■: 60% seed

pericarp caused no change in the pigment content till the 5th month of storage. A slight change can be seen in the remaining percent of the initial pigment content in the 7th and 9th months of storage. At 12 months of storage the residual percentage of the initial pigment content was higher in those samples which were added 40–60% ground seed (Fig. 1).

Since LEASE and LEASE (1956) demonstrated, that in a colour-stable variety the loss of colour content on aging was not correlated with the percentage of crude fat content, we can declare that cultivar Sz-20 is colour-stable variety. Probably the ester bonds of the main red components (capsanthin, capsorubin) are very stable in this variety and so less sensitive to autoxidation, therefore it retained 60% of the initial pigment content at the end of 12 months' storage (Fig. 1). The red components (capsanthin, capsorubin) of the total pigment content amounted 70% in all samples independently of the proportion of seeds. Organoleptic evaluation was performed too. Ground paprika without seeds (OM) retained the typical colour and taste characteristic of pure pericarp.

The red colour of ground paprika became deeper and fuller with the increase of seed content. Organoleptic sign of rancidity were not evident in samples even of 60% seeds.

However, other effect of the presence of seeds was observed. In the samples which contained 40 to 60% seed the remaining percentage of the initial pigment content is somewhat higher than in the other samples. According to DAOOD and BIACS (1986) this is due to the inhibitory or antioxidant effect of the seeds.

But there may be other explanations for this effect of the seeds, too. On the one hand the seeds can promote a better extraction of the pigment content. On the other, the oil in the seed may enclose the ground particles of the pericarp, and so protect them from the oxygen of air. The aim of these experiments was to clarify the effects of the added seeds upon the change of colour in ground red paprika (cultivar Sz-20). To find out the significance of the addition of seeds the results of the experiments were evaluated by analysis of variance, too. Two factors in three repetition were analysed taking into consideration the seven levels of addition of seed and nine levels of the storage period factor. According to this in case of seed additioning the F value calculated is 5.00 and in case of storage period the calculated F value is 27.55. Both values are higher than the appropriate degree of freedom belonging to P = 99.9% significance level related to F value in the synoptic table.

Both factors have significant effect on the change of the total pigment content of ground red paprika. For further analytical purposes we determined the SD value of individual factor and the mean value of level. The dates are in Tables 2 and 3.

Table 2

Detailed statistical analysis of the effect of added seed level

Ratio of added seed (%)	Mean value of level
0	80.04
10	82.04
20	78.83
30	81.90
40	85.56
50	83.26
60	87.22
$SD_{5\%}$	3.68

Table 3

Detailed statistical analysis of the effect of storage time

Month	Mean value of level
1	90.12
2	91.31
3	89.86
4	87.76
5	84.62
7	80.89
9	75.25
11	73.22
12	71.21
$SD_{5\%}$	4.18

It can be seen that the difference in the mean values between the samples containing 0% and 60% ground seed, respectively, is 7.18. This is about double of the SD value. Thus, the adding of ground seed to ground paprika pericarp is not detrimental to the pigment in ground red paprika, on the contrary, it seems to be useful.

During the whole storage period of 12 months the mean level varied from 90.12 to 71.21. The difference between the initial and final value amounted to 18.91, thus, the storage period is a very important factor of colour stability.

On the basis of our results we can say that cultivar Sz-20 is a colour stable paprika, and the oil content of the seeds has no detrimental effect upon the extracted pigment content, but the brightness of initial colour can be ascribed to the percentage of ground seed.

Sometimes a considerable loss of the initial pigment content occurs within six months of storage and this is attributable to other factors than the seed oil.

*

Grateful acknowledgement is due to Mrs E. Darázs manager of Paprika Processing Enterprise for supporting this work. The assistance of Mrs Zs. H. Horváth in carrying out analysis of variance and the technical assistance of Mrs K. Baici; is gratefully acknowledged.

Literature

Daood, H. & Biacs, P. A. (1986): Evidence for the presence of lipoxygenase and hydroperoxide-decomposing enzyme in red pepper seeds. *Acta Alimentaria, 15*, 307–318.

Hungarian Standard (1977): *Fűszerpaprika-őrlemény vizsgálata. Összes színezéktartalom meghatározása.* (Test methods for ground "paprika". Determination of total colouring matter content.) MSZ 9681/5-76.

Kanner, J., Harel Stella, Palevitch, D. & Ben-Gera, I. (1977): Colour retention in sweet red paprika (*Capsicum annuum* L.) powder as affected by moisture contents and ripening stage. *J. Fd Technol., 12*, 59–64.

Lease, J. G. & Lease, E. J. (1956): Factors affecting the retention of red color in peppers. *Fd Technol., 10*, 368–373.

Lease, J. G. & Lease, E. J. (1962): Effect of drying conditions on initial color, color retention, and pungency of red peppers. *Fd Technol., 16*, 104—106.

Nagy, J. & Murányi-Fekete Szücs, E. (1978): A fűszerpaprika termesztés és feldolgozás technológiai és mikrobiológiai vizsgálata. (Technological and microbiological examination of growing and processing of red pepper.) *Konzerv Paprikaipar, 6*, 210.

Pavisa, A. Cs., Hajdú, F., Hoschke, Á., Bodnár, J. & Biacs, P. (1987): High-performance liquid-chromatographic control of the standard specification of ground paprika. *Acta Alimentaria, 16*, 129–142.

Szabó, E. & Kosa, K. (1985): Fűszerpaprika színezőanyagtartalmának meghatározása. (Determination of the colour content in ground red pepper.) *Élelmiszerv. Közl., 31*, 154–160.

Vinkler, M. & Kiszel-Richter, M. (1972): A thin-layer-chromatographic method to determine the pigment content (components) in the pericarp of paprika. *Acta Alimentaria, 1*, 41—58.

Acta Alimentaria, Vol. 19 (1), pp. 87—104 (1990)

SEPARATION AND DETERMINATION OF D- AND L-AMINO ACIDS BY ION EXCHANGE COLUMN CHROMATOGRAPHY IN THE FORM OF DIASTEREOMER DIPEPTIDES

J.Csapó[a], B. Penke[b], I. Tóth-Pósfai[a] and Zs. Csapó-Kiss[a]

[a]University of Agriculture, Faculty of Animal Science, Kaposvár, H-7400 Kaposvár, Dénesmajor 2. Hungary
[b]Department of Medical Chemistry, Szent-Györgyi Albert University of Medicine, H-6722 Szeged, Dóm tér 8. Hungary

(Received: 6 June 1988; accepted: 28 February 1989)

A method of ion exchange column chromatography was developed for the determination of D- and L-amino acids in the form of diastereomer dipeptide. First the protein containing samples were hydrolyzed with 6 mol hydrochloric acid then the single amino acids were separated in an LKB automated amino acid analyzer with the LKB fraction collector. Following lyophilization the single amino acids were transformed into alanyl dipeptides with t-BOC-L-Ala-ONSu active ester and with $(t\text{-BOC})_2$-L-CySS-$(ONSu)_2$ active ester into 2-sulfonic acid-alanyl dipeptide after inserted oxidation by performic acid. The alanyl dipeptides were easily separated from one another and the initial amino acids. Determination of the D- and L-amino acids in this form is precise and accurate but takes some time (33–38 min). The 2-sulfonic acid-alanyl dipeptides appear on the chromatogram directly after cysteic acid. They separate easily from one another and from the cysteic acid formed from the excess of active ester. Accuracy of the determination is satisfactory. The coefficient of variation amounts to 4–10%.

The use of the method is suggested to laboratories having an amino acid analyzer and wish to determine D- and L-amino acids in synthetic amino acids complements, peptides or natural materials.

Keywords: D- and L-amino acid determination, diastereomer dipeptides, alanyl dipeptides, 2-sulfonic acid dipeptides, ion exchange column chromatography

Lately it became a more and more urgent requirement of our archeologist and stockbreeder colleagues to be able to separate and exactly determine D- and L-amino acids in different materials. In the last 25–30 years a great number of methods were developed by several authors to determine the age of fossils on the basis of amino acid racemization. The underlying principle of determination is the realization that L-amino acids forming the living organism, racemized after its death, are partly transformed into D-amino acids. Thus, determining the quantity of D- and L-amino acids e.g. in a fossil or a fossilized bone sample, taking into account various further factors (temperature, pH of the soil, moisture content, etc.) the age of the sample can be estimated. Our stockbreeder colleagues were incited to be interested in the determination of D- and L-amino acids by the following: with ruminant animal strains a part of the protein content of feed gets decomposed in the rumen and from the ammonia

forming the microorganisms present build up their own protein, thus a part of the protein is transformed into microbial protein. A new method of following the decomposition of feed protein in the rumen is the measurement of the D- and L-amino acid content, since D-alanin is formed only during microbiological protein synthesis, feed protein does not contain D-amino acids.

The amino acids most frequently used in the determination of the age of fossils are aspartic acid and D-allo-isoleucine and to follow up bacterial protein synthesis D-alanine. The determination from fossils of the other amino acids participating in protein building emerged as a requirement as well as the detection whether any other D-amino acid is formed beside D-alanine during bacterial protein synthesis, and of so whether these can be used to estimate the quantity of protein synthesized. These problems can be resolved only if there is a method available for the separation and determination of D- and L-amino acids. The method is described below.

Several methods have been developed for the separation and determination of amino acid enantiomers. To study racemization of the purified amino acids polarimetry and various enzymatic processes were applied.

One of the most rapid methods for the separation of D- and L-amino acids is gas chromatography. The enantiomers can be separated by forming a diastereomer pair using an appropriate asymmetrical reagent or the derivatives made volatile can be separated on an optically active stationary phase.

Lately the use of liquid chromatography for separation of enantiomers is gaining ground. WEINSTEIN and WEINER (1984) formed from amino acids fluorescent 5-dimethyl-aminonaphtalene-1-sulfonyl derivative and applying reversed phase liquid chromatography and N,N'-di-n-propyl-L-alanine and chiral copper acetate pack, could separate all the D- and L-enantiomers of the amino acids forming the protein. MARFEY (1984) produced with the help of 1-fluoro-2,4-dinitrophenyl-5-L-alanine-amid, containing a highly reactive fluor atom, diastereomer derivatives, which can be separated by liquid chromatography.

Of the protein building acids beside hydroxyproline the isoleucine has two asymmetric centers. The D-allo-isoleucine formed from isoleucine in the course of time, being the diastereomer of isoleucine, appears in the chromatogram between isoleucine and methionine during routinly applied ion exchange amino acid separation, yields a well separated and well evaluable peak. Racemization of the carbon atom in the alfa position and D-allo-isoleucine formation during peptide synthesis were thoroughly investigated by BODANSZKY and CONKLIN (1967). MANNING and MOORE (1968) described also an ion exchange column chromatography technique for the separation of D- and L-amino acids and their quantitative determination. The method is suitable for the quantitative determination of trace amounts of D-amino acids beside L-amino acid. The essence of the method is the reaction occurring between an L-amino acid

N-carboxyanhydride and the D- and L-amino acids to be investigated. In the course of this reaction diastereomer dipeptides are formed, suitable for separation by ion exchange. To produce dipeptides the method described by HIRSCHMANN and co-workers (1967) was applied, in the course of which N--carboxy-alfa-amino acid anhydride was added to the amino acid to be tested in an aqueous medium of 0–2 °C, pH 10 in the range of 10.2–10.4.

By comparing the information of the related literature to the possibilities in our laboratory we decided, taking into account the developments of recent years in peptide chemistry, to develop an ion exchange column chromatographic method for the separation of D- and L-amino acids in the form of diastereomer dipeptides. In the development of the method we kept in view that the experiments described could be reproduced in a laboratory provided with an amino acid analyzer. The method should consist of simple steps and suitable for routin tests. Allowing for the above the method we suggest for the separation and determination of D- and L-amino acids consists of the steps as given below:

— preparation of the sample;
— hydrolysis of the protein in the sample with hydrochloric acid;
— separation of the amino acids by ion exchange column chromatography;
— synthesis of the diastereomer dipeptides;
— separation and determination of the diastereomer dipeptides.

1. Materials and methods

1.1. Materials

The most important step in our method is the synthesis and separation of diastereomer dipeptides. The generally applied method for peptide synthesis in homogeneous solutions is active ester condensation. This reaction is nearly quantitative, the purification of the product is simple therefore it was decided to use it. Taking into consideration that the separation of the amino acids to be tested as well as the separation of the diastereomer dipeptides occurs in aqueous medium we chose of the active esters N-hydroxy-succinimide ester (ONSu). These esters get connected extremely well in aqueous medium and the active ester formed as by-product does not interfere with amino acid analysis. Next decision had to be taken on the ground applied to protect the amino group of the acylating amino acid in the course of active ester condensation. Since in L-L and D-L dipeptide determination in the amino acid analyzer the protecting group has to be removed in order to make the compound to be

measured ninhydrin positive, we chose a tercier butyl-oxy-carbonyl group (BOC), partly because the BOC group is easy to build up, partly because after dipeptide synthesis the cleaving off the protecting group with trifluor acetic or 1 mol l^{-1} glacial acetic acid containing hydrochloric acid can be simply carried out.

Subsequent to the selection of the protecting group and the active ester the selection of the acylating amino acid had to be decided of among the amino acids building the protein. Since the acylating amino acid has to have asymmetry center and connecting has to take the shortest possible time, our choice fell on alanine (Ala). The Ala-X dipeptides (X = protein building D- or L--amino acid) — as has been found in the experiments aimed at separating them — even in the case of aspartic acid, appear in the chromatogram after Ala, thus, separation takes at least 1–1.5 h. We tried to find a way to make the synthesized diastereomer dipeptides appear in a shorter time on the chromatogram. Therefore, we selected as the second acylating amino acid cystine (CySS) hoping that after the active ester condensation a tripeptide is formed which will break down after oxidation with performic acid into two dipeptides. One of the two peptides, cysteic acid accelerates the elution of the dipeptide thereby significantly shortening the time needed to separate the diastereomer dipeptides.

We synthesized the tercier-butyl-oxy-carbonyl-L-alanine-N-hydroxy--succinimide ester (t-BOC-L-Ala-ONSu) and the bis-tercier-butyl-oxycarbonyl--L-cystine-bis-N-hydroxy-succinimide ester $(t\text{-}BOC)_2$-L-CySS-$(ONSu)_2$ with the intention of using the first one to determine the diastereomer dipeptides of the acidic amino acids, while the second of the neutral or basic amino acids. The protected active ester of the two amino acids was synthesized as described by BAJUSZ (1980).

Subsequent to the synthesis of the active esters diastereomer dipeptides were prepared from crystallized amino acids (standards) or from the single amino acids separated in the amino acid analyzer.

1.2. Separation of the protein building amino acids

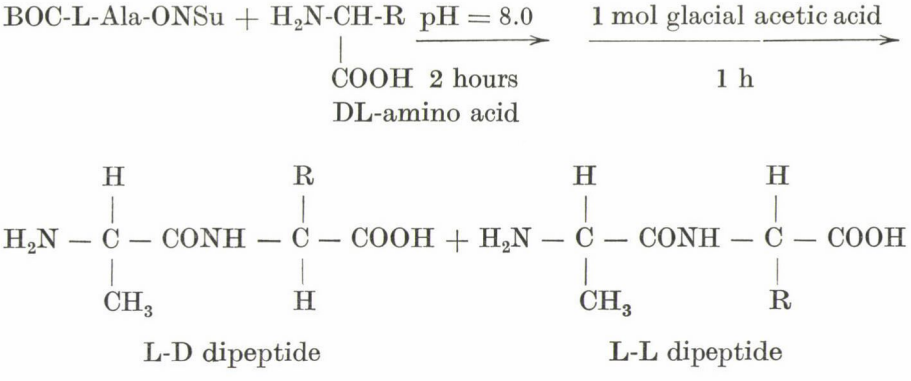
The raw protein content of the bone samples or the rumen liquid was determined with a Kjel-Foss 16200 rapid nitrogen analyzer and then depending on the raw protein content 100–1000 mg (equal to ca 10–20 mg protein) material was hydrolyzed with 6 mol l^{-1} hydrochloric acid for 24 h. When hydrolysis was finished the hydrochloric acid was removed by lyophilisation and the silicates separating during dissolution in water were removed by centrifuging from the solution containing free amino acid. Thus, the sample is ready for the determination of isoleucine and D-alloisoleucine by ion exchange column chromatography or by the same method for the separation of the protein build-

ing amino acids. Determination and separation have been carried out with LKB 4101 type amino acid analyzer and the connected LKB fraction collector. The test-tubes corresponding to individual amino acids were identified and lyophilised to the dry. Then the diastereomer dipeptides were produced from the individual amino acids or from the mixture of several amino acids.

1.3. Synthesis of diastereomer dipeptides

The synthesized amino acid or the residue separated with the amino acid analyzer and dried by lyophilisation was dissolved in water to obtain a solution of 1–10% concentration for everyone of the amino acids. The pH of the solution was set at pH 8 by adding 1–2 sodium hydrogen carbonate crystal, then the protected active ester of Ala or CySS, dissolved in dioxane–water 1 : 1 was added in 2 to 2.5 times excess. In the case of Ala the reaction mixture was shaken for 2 h in a shaking apparatus and in the case of CySS for 8 h at room temperature, this was followed by drying for 2 or 8 h, respectively, then lyophilized to dry. After drying the protective BOC group was cleaved off in both cases with 1 mol glacial acetic acid solution (reaction time 1 h), finally lyophilization was repeated. The alanyl dipeptides were then dissolved in citrate buffer of pH = 2.2. Appropriately diluted the solution was then applied to the ion exchange column of the amino acid analyzer to separate the diastereomer dipeptides.

The reactions are summarized as follows:



The cystinyl dipeptide was oxidized with performic acid according to HIRS (1956). After breaking down the disulfide bridge two dipeptides containing cysteic acid were obtained. The performic acid was removed, the residue was dissolved in citric acid buffer of pH 2.2 and the solution was applied to the ion exchange column of the analyzer. Concentrations were set by dilution so that the dipeptides formed should fall between 50 and 100 nanomol.

The reaction equations are as follows:

$$(BOC)_2\text{-L-CySS-}(ONSu)_2 + H_2N{-}\underset{\underset{COOH}{|}}{CH}{-}R \xrightarrow[8\ h]{pH = 8.0} \xrightarrow[1\ h]{1\ mol\ glacial\ acetic\ acid}$$

DL amino acid

$$\xrightarrow[\text{performic acid}]{\text{oxidized by}}$$

$$H_2N - \underset{\underset{CH_2}{|}\\ \underset{SO_3H}{|}}{\overset{\overset{H}{|}}{C}} - CONH - \underset{\underset{H}{|}}{\overset{\overset{R}{|}}{C}} - COOH + H_2N - \underset{\underset{CH_2}{|}\\ \underset{SO_3H}{|}}{\overset{\overset{H}{|}}{C}} - CONH - \underset{\underset{R}{|}}{\overset{\overset{H}{|}}{C}} - COOH$$

L-D dipeptide L-L dipeptide

1.4. Separation of the diastereomer dipeptides

The alanyl dipeptides produced by the reaction as described in 1.3 or the sulfonic acid-alanyl-dipeptides obtained by performic acid oxidation from the N,N'-bis-AA-yl-cystine tripeptide were separated and determined in the LKB 4101 type automated amino acid analyzer.

1.4.1. Separation and determination of alanyl dipeptides. The separation of L-L or D-L diastereomer dipeptides formed from D-Asp, DL-Glu, DL-Ala, DL-Val, DL-Ile, DL-Phe amino acids is shown in Figs. 1, 2, 3, 4, 5 and 6, respectively. Conditions of separation were as follows:

Instrument	LKB Biochrom
Dimensions of the column	500×6 mm
Ion exchange resin	Chromex UA-8
Flow rate of buffer	80 $cm^3\ h^{-1}$
Flow rate of ninhydrin	40 $cm^3\ h^{-1}$
Column temperature	50 °C for 60 min, then
	70 °C till the end of analysis
Buffer A: pH 3.25	Na molarity = 0.2; 25 min
Buffer B: pH 4.25	Na molarity = 0.2; to the end of analysis
Sodium hydroxide	= 0.4 mol; 15 min
Equilibration:	Buffer A; 45 min

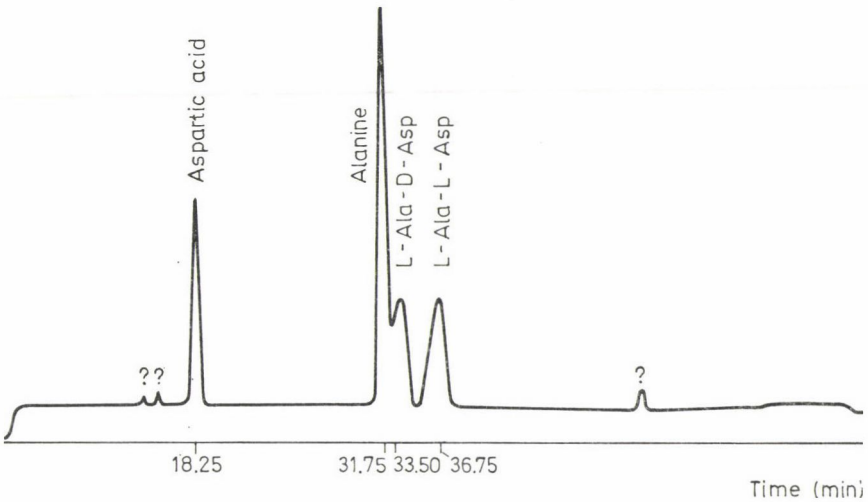


Fig. 1. Determination of D- and L-aspartic acid

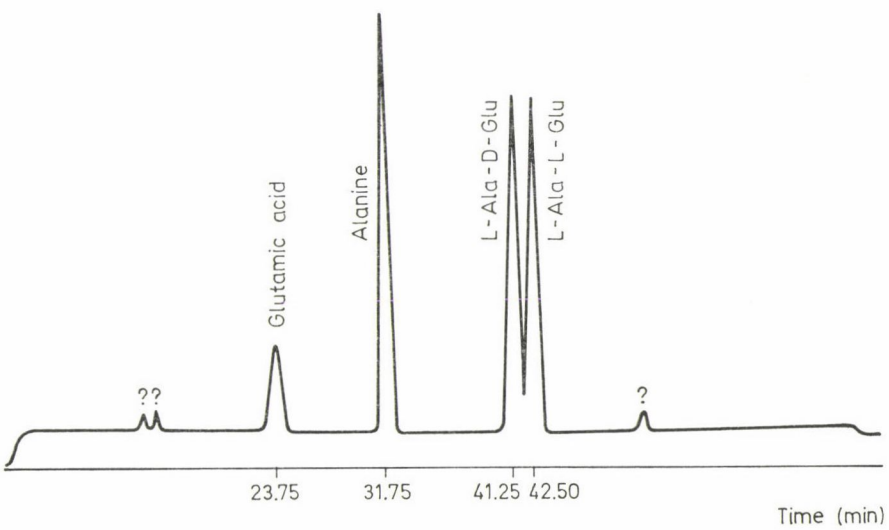


Fig. 2. Determination of D- and L-glutamic acid

On evaluating the chromatograms it was established that in each case 5 well separated ninhydrin positive peaks were formed. The peaks represent; the initial amino acid, the alanyl utilized as active ester, the L-Ala-L-AA dipeptide, the L-Ala-D-AA dipeptide and at the end of the chromatogram ammonia. In chromatogram 1 it can be seen that under the experimental conditions L-Ala-L-Asp appears as the shoulder peak of Ala, however, the separation of the two diastereomer dipeptides is very satisfactory. Separation

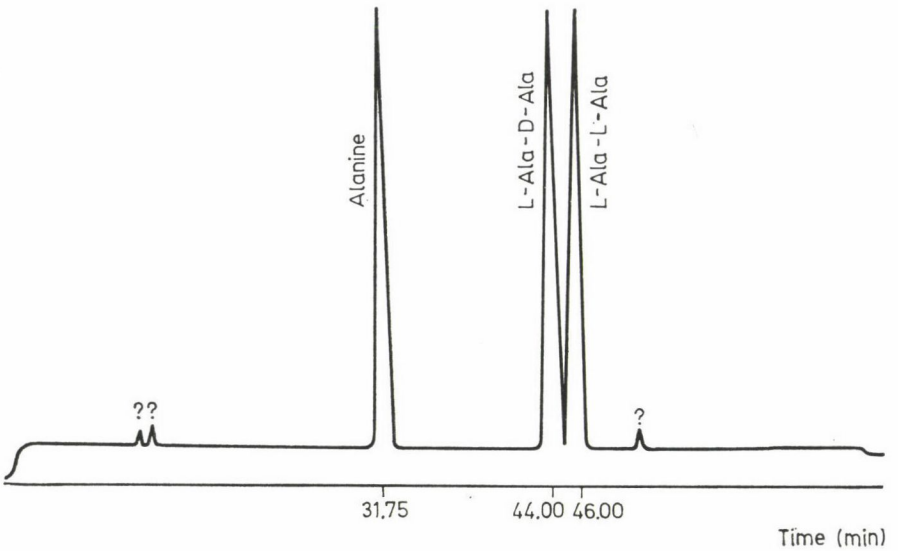


Fig. 3. Determination of D- and L-alanine

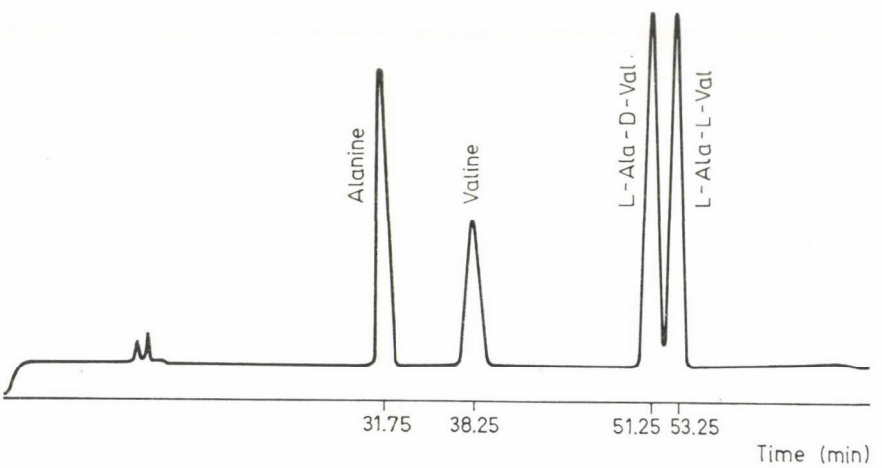


Fig. 4. Determination of D- and L-valine

is very satisfactory in the case of Glu, Ala, Val, Leu and Phe, too. Separation and evaluation are not disturbed by other ninhydrin positive compounds present. In addition to the chromatograms shown separation was satisfactory also in the case of alanyl diastereomer dipeptides for Pro, Met, Ile and Tyr. Thr and Ser separate as twin peaks. Experiments with the basic amino acids are in progress at present.

The optimum conditions of diastereomer alanyl dipeptide analysis (tem-

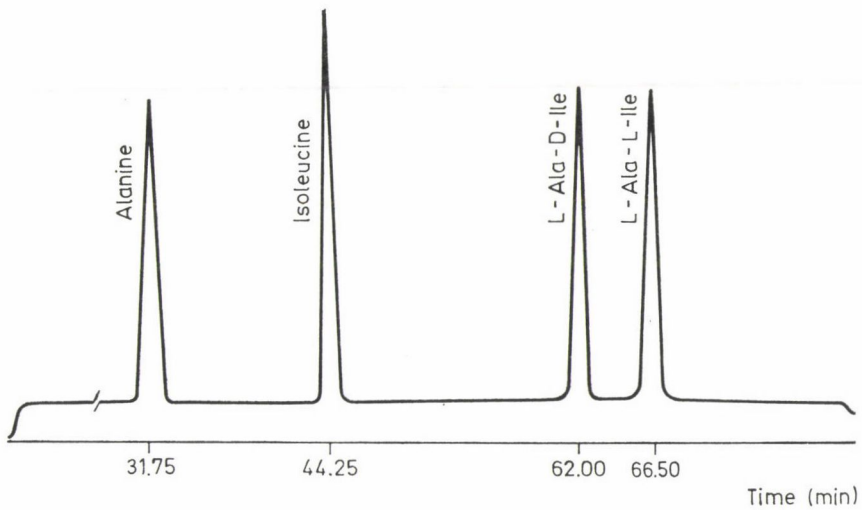


Fig. 5. Determination of D- and L-isoleucine

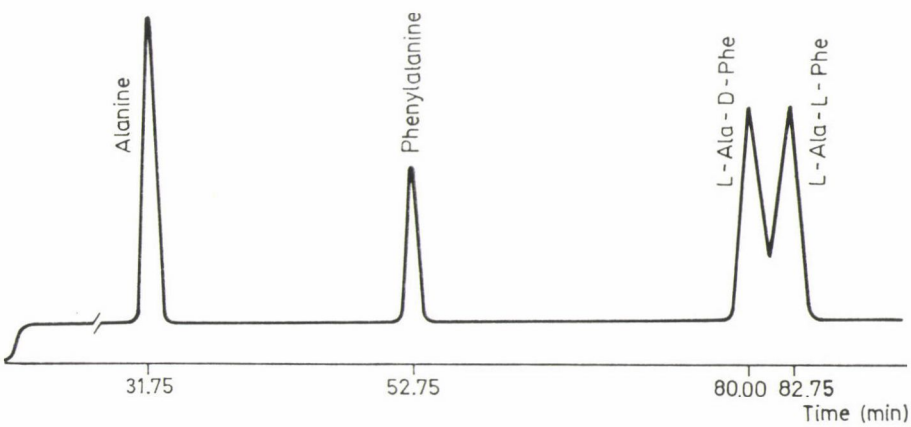


Fig. 6. Determination of D- and L-phenylalanine

perature, pH) as well as the position of peaks in the chromatograms are summarized in Table 1. Table 2 contains data of the initial materials and of the amino acids to be analyzed.

The tables show that the amino acids and diastereomer dipeptides can be sorted out on the basis of the position of their peaks. Those amino acids which do not disturb one another's separation belong to the same group and can be determined in a single step. The groups are as follows:

— Asp, Ser, Pro, Ala
— Thr, Glu, Ala
— Ala, Val, Ile
— Ala, Met, Leu

Figure 7 shows the separation of the diastereomer dipeptides obtained from the amino acids Thr, Glu and Ala, while Fig. 8 those from Val and Ile.

Table 1

Conditions of chromatography and the location of diastereomer dipeptides in the chromatogram

Dipeptide	Temperature of isolation (°C)	Appearence of peak (min)	Location of peak in relation to Ala (Ala = 1.00)
L-Ala-D-Asp	50	33.50	1.05
L-Ala-L-Asp	50	36.75	1.16
L-Ala-D-Thr	50	36.25	1.14
L-Ala-L-Thr	50	37.25	1.17
L-Ala-D-Ser	50	38.50	1.21
L-Ala-L-Ser	50	39.75	1.25
L-Ala-D-Glu	50	41.25	1.30
L-Ala-L-Glu	50	42.50	1.34
L-Ala-D-Pro	50	42.00	1.32
L-Ala-L-Pro	50	43.75	1.38
L-Ala-D-Ala	55	44.00	1.39
L-Ala-L-Ala	55	46.00	1.45
L-Ala-D-Val	60	51.25	1.61
L-Ala-L-Val	60	53.25	1.68
L-Ala-D-Met	65	54.25	1.71
L-Ala-L-Met	65	56.25	1.77
L-Ala-D-Ile	70	62.00	1.95
L-Ala-L-Ile	70	66.50	2.09
L-Ala-D-Leu	70	64.25	2.02
L-Ala-L-Leu	70	68.00	2.14
L-Ala-D-Tyr	70	76.25	2.40
L-Ala-L-Tyr	70	78.50	2.47
L-Ala-D-Phe	70	80.00	2.52
L-Ala-L-Phe	70	82.75	2.61

Table 2

Location of the initial amino acids in the chromatogram

Amino acid	Appearance of peak (min)	Amino acid	Appearance of peak (min)
Asp	18.25	Val	38.25
Thr	20.25	Met	42.00
Ser	21.25	Ile	44.25
Glu	23.75	Leu	45.75
Pro	25.25	Tyr	51.25
Ala	31.75	Phe	52.75

As it can be seen, separation is satisfactory, peaks can be evaluated, thus, quantitative determination is in no way hindered.

1.4.2. Separation and determination of 2-sulfonic acid alanyl dipeptides. Figure 9 shows the chromatogram of the DL-Asp, Fig. 10 the DL-Glu, Fig. 11 the DL-Ala, Fig. 12 the DL-Val, Fig. 13 the DL-Ile and Fig. 14 the $(BOC)_2$ L-CySS-$(ONSu)_2$ active ester after preparation as described above. Conditions of separation differ from those described in para. 1.4.1. as follows:

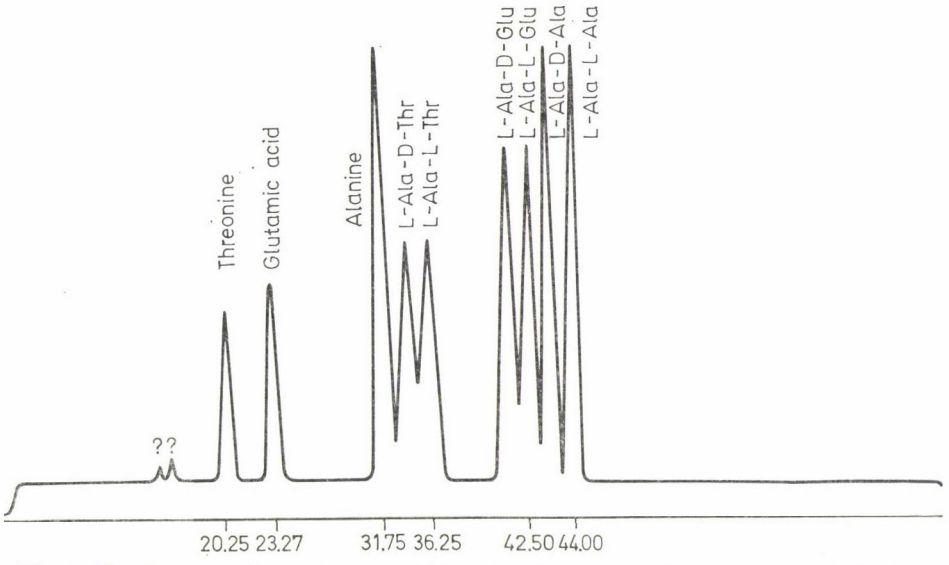


Fig. 7. Simultaneous determination of D- and L-threonine, glutamic acid and alanine

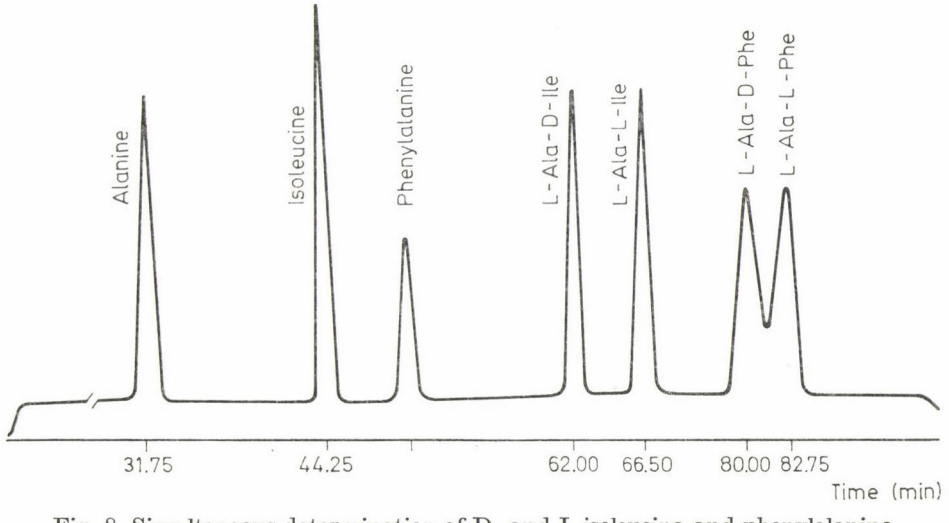


Fig. 8. Simultaneous determination of D- and L-isoleucine and phenylalanine

Temperature of column: 40 °C during the whole process of separation
Buffer A: pH 2.90; Na molarity = 0.2; to end of analysis
Na-hydroxide = 0.4 mol; 15 min
Equilibration: Buffer A (pH 2.9); 45 min

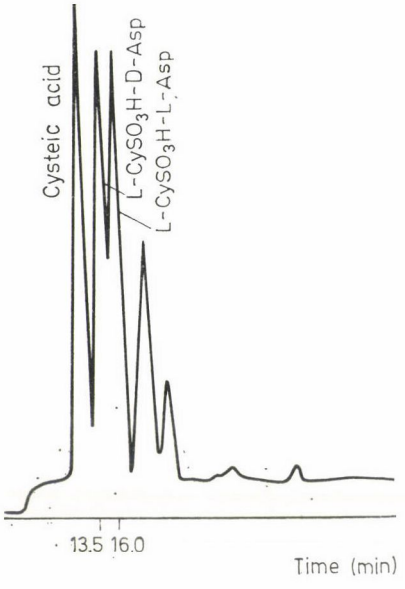


Fig. 9. Determination of D- and L-aspartic acid

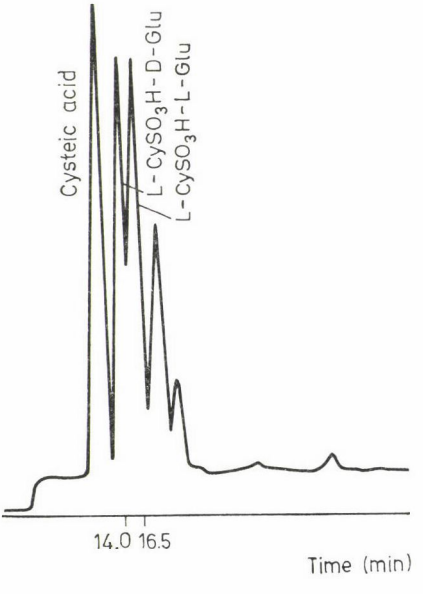


Fig. 10. Determination of D- and L-glutamic acid

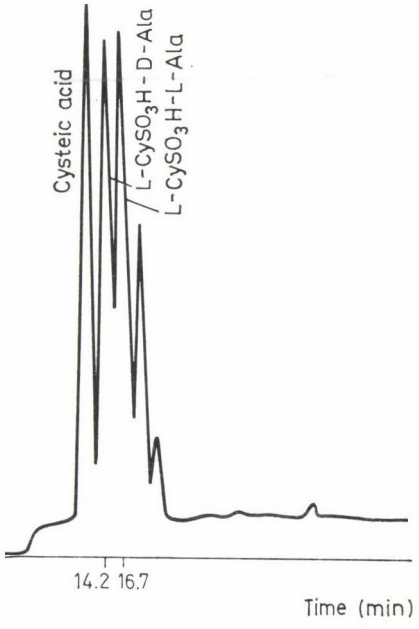


Fig. 11. Determination of D- and L-alanine

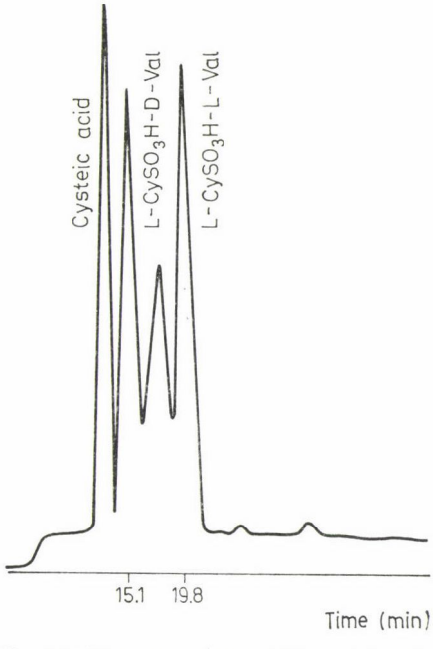


Fig. 12. Determination of D- and L-valine

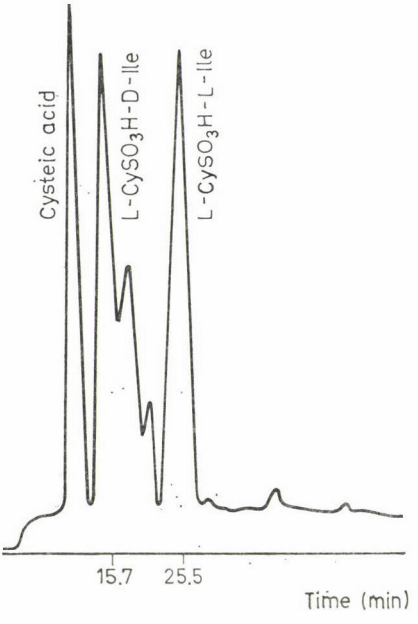


Fig. 13. Determination of D- and L-isoleucine

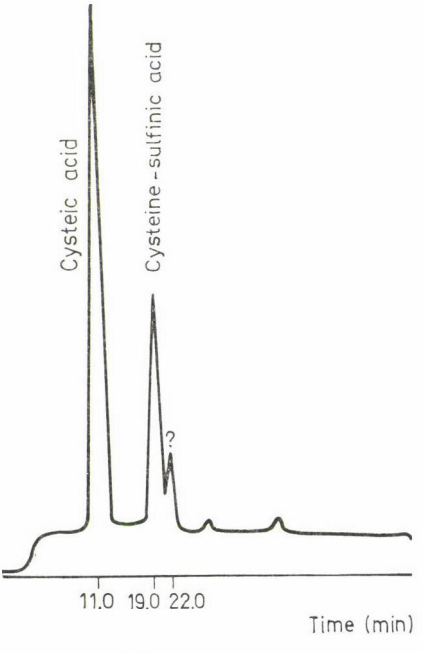


Fig. 14. Chromatogram of the active ester subsequent to oxidation

In the chromatogram cysteic acid appears in the 11th min directly after the front and is followed by two peaks, one of them in the 19th min forming about 25% of the cysteic acid peak and the second in the 22nd min forming about 5% of the cysteic acid peak. The higher peak is probably that of cysteine-sulfinic acid, while the smaller peak could not be identified so far.

These two peaks do not cause any difficulty in relation to Asp, Thr, Ser, Glu, Pro or Ala because the two diastereomer 2-sulfonic acid-alanyl dipeptides formed from the above amino acids appear in the chromatogram in the free space between cysteic acid and the second peak. The separation of the diastereomer dipeptides from cysteic acid is satisfactory, it can be compared to the separation of Thr-Ser in normal amino acid analysis. The first methodological difficulty arises in relation to valine where the separation of L-L dipeptide peak and the peak ascribable to $CySO_2H$ is not perfect, they partly overlap. This problem gets modified in the case of Ile so that the separation from L-L dipeptide is perfect, while the peak of $CySO_2H$ merges without causing trouble, into that of D-L-dipeptide. The situation is similar with isoleucine, too. In the case of tyrosine the problem modifies so that the D-L-dipeptide, appearing in the chromatogram first is directly after the two peaks and in the case of Phe nothing interferes with separation. Since performic acid oxidizes not only cysteine but methionine as well producing methionine sulfone, the 2-sulfonic acid-alanyl-methionine-sulfone dipeptide, similarly to aspartic acid appears directly after cysteic acid in the chromatogram. In this case special care has to be taken to finish oxidation by performic acid completely, otherwise a mixture of the dipeptide of methionine sulfone and methionine sulfoxide is obtained in which case determination is almost impossible.

Since during the retention period of 2-sulfonic acid-alanyl-dipeptides differences are only slight for the consecutive amino acids, it is not possible to produce amino acid groups similar to those described in para. 1.4.1. The advantage of this method in comparison to the former one is that the determination of the D- or L-isomer of any one amino acid takes much less time than that of the alanyl dipeptides. On the other hand, it is disadvantageous that the D- or L- isomer of only a single amino acid can be determined at a time.

2. Results

2.1. Accuracy of the determination of the optical isomers of amino acids in the form of diastereomer dipeptides

After finishing the development of the method the D- and L-amino acid composition of synthesized amino acid mixtures was determined. Results were summarized in Table 3. Similarly to those presented in the table the analysis of

Table 3

Determination of the D- and L-amino acids in various mixtures in the form of alanyl diastereomer dipeptides

Material tested	Theoretical value (%)		Value measured (%)		Number of measurements	Standard deviation		Coefficient of variation	
	D	L	D	L		D	L	D	L
Glutamic acid	50	50	48.2	51.8	5	1.59	1.61	3.30	3.11
	25	75	26.4	73.1	5	0.97	2.11	3.67	2.89
	5	95	5.8	94.3	5	0.21	2.62	3.62	3.84
	1	99	0.95	98.7	5	0.043	2.74	4.53	2.78
Alanine	50	50	51.0	49.3	5	1.62	1.58	3.18	3.21
	25	75	25.2	75.1	5	1.05	2.05	4.17	2.73
	5	95	4.6	94.3	5	0.24	2.74	5.22	2.91
	1	99	1.10	98.8	5	0.055	2.90	5.00	2.94
Valine	50	50	51.1	49.7	5	1.74	1.63	3.41	3.28
	25	75	23.9	76.1	5	1.08	2.14	4.25	2.81
	5	95	5.1	93.9	5	0.26	2.81	5.10	2.99
	1	99	1.06	99.1	5	0.062	2.91	5.85	2.94
Isoleucine	50	50	50.1	51.1	5	1.69	1.72	3.37	3.37
	25	75	25.6	74.8	5	0.99	2.23	3.87	2.98
	5	95	4.9	94.6	5	0.32	2.62	6.53	2.77
	1	99	0.98	98.9	5	0.051	2.84	5.20	2.87

every amino acid was carried out in 5 parallels in each concentration, however, only a few of them are discussed.

As it can be seen in the table, the coefficient of variation decreases with increasing concentration, thus at higher concentrations the accuracy of determination is higher. However, even at the lowest concentration the value of the coefficient of variation does not reach 10, thus the method is reliable, its reproducibility is satisfactory.

2.2. Accuracy of the determination of optical amino acid isomers in the form of diastereomer 2-sulfonic acid-alanyl dipeptides

In Table 4 the results of experiment with 2-sulfonic acid-alanyl dipeptides are presented. As it can be seen, the mean values of the theoretical results and those determined are practically identical, the standard deviations, however, are much higher than those given in Table 3. Probably one of the reasons for this lies in the fact, that the production of 2-sulfonic acid-alanyl dipeptides requires one more step (performic acid oxidation) than that of alanyl dipeptides. Another reason may be that the isolation of the diastereomer dipeptides of very short retention time is not as perfect as with alanyl dipeptides. Finally the third reason may be that cysteic acid, cystein sulfinic acid and the hitherto not identified small peak may occasionally disturb the determination of sulfonic acid containing dipeptides.

Table 4

Determination of the D- and L-amino acids in various mixtures in the form of 2-sulfonic acid-alanyl-diastereomer dipeptides

Material tested	Theoretical value (%)		Value measured (%)		Number of measurements	Standard deviation		Coefficient of variation	
	D	L	D	L		D	L	D	L
Glutamic acid	50	50	51.7	48.2	5	3.02	2.54	5.84	5.27
	25	75	25.3	75.1	5	1.94	3.22	7.67	4.29
	5	95	4.8	94.9	5	0.51	4.85	10.63	5.11
	1	99	0.99	99.2	5	0.092	4.99	9.29	5.03
Alanine	50	50	49.9	51.0	5	2.98	2.63	5.97	5.16
	25	75	24.6	74.9	5	2.00	3.11	8.13	4.15
	5	95	5.1	95.2	5	0.54	4.62	10.59	4.85
	1	99	1.02	98.4	5	0.085	5.03	8.33	5.11
Valine	50	50	50.3	48.9	5	2.79	2.71	5.55	5.54
	25	75	24.7	75.3	5	2.11	3.33	8.54	4.42
	5	95	4.89	95.2	5	0.48	4.71	9.82	4.95
	1	99	1.11	98.7	5	0.091	5.11	8.20	5.18
Isoleucine	50	50	48.7	49.9	5	2.94	2.48	6.04	4.97
	25	75	25.3	74.8	5	1.85	3.01	7.31	4.02
	5	95	5.11	95.2	5	0.47	4.90	9.20	5.15
	1	99	0.97	98.8	5	0.101	4.97	10.41	5.03

The value of the coefficients of variation exceeds 10 in 3 cases only and even then very slightly. In every other case it is below 10. Thus, this method is also reliable and its reproducibility is satisfactory.

3. Conclusions

In spite of the fact that the highly efficient liquid-chromatographic methods gain in popularity in the isolation and determination of optically active compounds, conforming to the actual conditions in our laboratory and the about 30 laboratories carrying out feed analyses and being provided with amino acid analyzer, we developed an ion exchange column-chromatographic method for the quantitative determination of D- and L-amino acids.

Data in Tables 1 and 3 show that t-BOC-L-Ala-ONSu is highly suitable for the synthesis of diastereomer dipeptides. After connection it is easy to remove the t-BOC group, the alanyl dipeptides are easily isolated, standard deviations of the method are low and in the majority of cases the coefficients of variation are below 5. By appropriate grouping of the amino acids it becomes possible to determine the D- and L-isomers of several (two or three) amino acids in a single step.

In order to accelerate the method (to be able to elute the diastereomer dipeptides in less time) it seemed more expedient to synthesize a dipeptide

containing an acidic amino acid. Knowing the difficulties involved in protecting and later freeing the amino, carboxyl and hydroxy groups our choice has fallen on cysteic acid appearing directly after the front. The N-hydroxy-succinimide ester of cystine, protected by t-BOC group was connected to the amino acid to be determined. Then the t-BOC group was removed and the cystine was oxydized by performic acid into cysteic acid and thus were the 2-sulfonic acid--alanyl-diastereomer dipeptides obtained. These dipeptides appeared — as expected — directly behind cysteic acid in the chromatogram and in most cases they were easy to isolate from cysteic acid as well as from one another. The advantage of this method over the former one lies in its rapidity. An analysis takes hardly 20–25 min, however, it is more labour intensive and standard deviations, particularly at lower concentrations, are nearly twice as high (Table 4). Despite of the difficulties as recounted above, both methods are suitable for the detection of at least 1% D- (or L-) amino acid in the presence of 99% L- (or D-) amino acid. The use of the method is suggested to all the laboratories which are in the possession of an amino acid analyzer and want to determine the D- and L-amino acids in synthetic amino acids, peptides or natural substances.

*

The authors are highly indebted to Mr R. FERENCZY, Medical Chemistry Institute of the Szent-Györgyi Albert Medical University Szeged, for the help rendered in producing the protected active esters.

The financial help provided by the Protein Program Bureau of the National Technical Development Committee, Budapest, is also highly appreciated.

Literature

BAJUSZ, S. (1980): Peptidszintézis. (Peptide synthesis.) — in: CSÁKVÁRI, B. (Ed.) *A kémia újabb eredményei.* 47. (New results in chemistry, Vol. 47.) Akadémiai Kiadó, Budapest, pp. 230.

BODANSZKY, M. & CONKLIN, L. E. (1967): A simple method for the study of racemization in peptide synthesis. *Chem. Commun., 556,* 773–774.

HIRS, C. H. W. (1956): The oxidation of ribonuclease with performic acid. *J. biol. Chem., 219,* 611–621.

HIRSCHMANN, R., STRACHAN, R. G., SCHWAM, E. F., SCHOENEWALDT, H., JOSHUA, B., BARKEMEYER, B., VEBER, D. F., PALEVEDA, W. J., JACOB, T. A., BEESLEY, T. E. & DENKEWALTER, R. G. (1967): The controlled synthesis of peptides in aqueous medium III. Use of Leuchs' anhydrides in the synthesis of dipeptides. Mechanism and control of side reactions. *J. org. Chem., 32,* 3415–3425.

MANNING, J. M. & MOORE, S. (1968): Determination of D- and L-amino acids by ion exchange chromatography as L-D and L-L dipeptides. *J. biol. Chem., 243,* 5591–5597.

MARFEY, P. (1984): Determination of D-amino-acids. — Part II. Use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene. *Carlsberg Res. Commun., 49,* 591–596.

WEINSTEIN, S. & WEINER, S. (1984): Enantiomeric analysis of a mixture of the common protein amino acids and their Dns derivatives. *J. Chromat., 303,* 244–250.

Acta Alimentaria, Vol. 19 (2), pp. 105 (1990)

BOOK REVIEW

ASEPTIC PACKAGING OF FOOD

H. REUTER (Ed.)

Technomic Publishing Co., Inc., Lancester-Basel, 1989, 269 pages

A comprehensive guide and reference to aseptic food packaging technology is available in this book. It contains papers presented by 20 leading authorities on the subject at the Symposium organized by Behr's Seminar. The volume is an English-language translation of a book originally published as *Aseptisches Verpacken von Lebensmitteln* in the Federal Republic of Germany.

A well-illustrated text covers the principles and modern international practices of aseptic packaging of sterile products like milk and homogeneous dairy products; fruit and vegetable juices, soups, sauces, as well as of non-sterile foods and non-homogeneous products with smaller or larger solid particles.

The book contains six chapters. After the introduction of aseptic packaging of foods it introduces the presterilization of homogeneous low viscosity foods and liquid foods containing particles. The third topic includes the microbiological principles for aseptic packaging. In the next well-detailed chapter the aseptic filling and packaging systems, the design and function of filling and packaging machinery and equipment are discussed. The fifth part is for packaging materials for aseptic packaging and the topic of the last chapter introduces the aseptic processing in the food industry. The readers can find the list of references at the end of chapters.

This publication is not intended as an academic textbook but rather as a reference book for practitioners in the field, primarily professionals of the food industry: managers, food technologists, chemists and engineers working in production, controlling, research and development. It should be of interest to producers of packaging materials and of aseptic filling and packaging machinery, furthermore to manufacturers of plants for presterilization of products.

I. VARSÁNYI

PRINTED IN HUNGARY

Akadémia Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Introduction of chicken irradiation on an industrial scale
DÖLLSTÄDT, R., GRAHN, CHR., HÜBNER, G., KÖHLER, B. & KRAUTSCHICK, J.

Investigation of the state of water in fibrous foodstuffs by near infrared spectroscopy
KAFFKA, K. J., HORVÁTH, L., KULCSÁR, F. & VÁRADI, M.

New phase diagram of the D-glucose water system
SMELIK, A., TÖRÖK, SZ. & VUKOV, K.

Investigation of the relationships between wheat lipids and baking properties
KÁRPÁTI, E. M., BÉKÉS, F., LÁSZTITY, R., ÖRSI, F., SMIED, I. & MOSONYI, Á.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 19 No. 1 — 1990

CONTENTS

Index: 26.039

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 19 **June 1990** **Number 2**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1990: Volume 19 (4 issues).
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 19

1990

AKADÉMIAI KIADÓ
BUDAPEST

PRINTED IN HUNGARY

Akadémia Kiadó és Nyomda Vállalat, Budapest

Acta Alimentaria, Vol. 19 (2), pp. 107—124 (1990)

INTRODUCTION OF CHICKEN IRRADIATION ON AN INDUSTRIAL SCALE

R. DÖLLSTÄDT[a], CHR. GRAHN[a], G. HÜBNER[a], B. KÖHLER[b] and J. KRAUTSCHIK[c]

[a] Central Institute of Isotope and Radiation Research of the Academy of Sciences, Permoserstrasse 15, Leipzig, DDR-7050. German Democratic Republic
[b] County Institute of Veterinary Research, Pappelallee 2, Potsdam, DDR—1510. German Democratic Republic
[c] Veterinary Hygiene Inspection Halle, Regional Department, Wittenberg — 4600. German Democratic Republic

(Received: 24 February 1988; revision received: 29 June 1989; accepted: 11 August 1989)

Starting from international research results and the necessity for Salmonella-inactivation in chicken carcasses in the GDR, a research project was devised to establish the optimum radiation dose, to characterize sensorial impairment and to work out a technology for treatment in the AGRORAD multi-purpose industrial scale irradiation plant, thus serving public health and avoiding losses for the manufacturers. A benefit/cost ratio was estimated.

Keywords: chicken; cobalt-60; cost benefit analysis; irradiation; microbiology; radiation technology; Salmonella

In general diseases caused by food-stuffs are increasing on a world scale, the number of internationally registered salmonelloses having doubled within the last 16 years. In animal production and processing chicken carcasses are the most heavily Salmonella-contaminated line of production.

Fighting salmonellosis in chicken involves in the first place hygienic measures. As these, however, will always show gaps, the use of ionizing radiation is a potential method for the decontamination of Salmonella-infected chicken carcasses.

Killing Salmonellae in eviscerated chicken by ionizing radiation is — according to various international boards (FAO, IAEA, WHO) — the only possibility for efficiently combating Salmonellae while at the same time preserving all the sensorial properties.

On the basis of intensive research within international projects such as the long-term KARLSRUHE project in 1976, the recommendation was made by an expert commission of the FAO, IAEA, WHO for the use of radiation treatment for eviscerated chicken at a dose between 2 and 7 kGy.

According to a study by MORRISON and ROBERTS (1985) the radiation treatment of 81% of the chicken carcasses in the USA at a dose of 2.5 kGy led to a benefit/cost ratio of 2.2 to 4.2 and an annual net profit of $ 186 to 498 million.

The most comprehensive and longest study on the wholesomeness of irradiated food, particularly chicken carcasses, was carried out by Raltech

Scientific Service (commissioned by the Army Medical Department) (WIEBRICKI & JOSEPHSON, 1986) between 1976 and 1986. The research results show that eviscerated chicken irradiated at 58 kGy, i.e. 19 times the dose of that envisaged in the US (2.5–3 kGy) did not cause any health damage when fed to test animals (according to MCGOWN et al., 1981; DAHLGREEN et al., 1982), which is repeated evidence of the wholesomeness of irradiated food, and chicken in particular. This study formed the essential basis for the Food and Drug Administration in making the necessary decisions.

In the GDR there are about 2000 t of Salmonella-contaminated chicken carcasses every year, which are labelled "fit after treatment". At present these carcasses are processed for canned food. Due to limited capacities for this type of processing, such carcasses are at present in cold storage. It is, however, necessary to use them in their nature form and, at the same time, to regain cold-storage capacity.

The aim of our research project was to establish the radiation dose required for the inactivation of Salmonellae in such carcasses, to characterize the sensorial influences possibly exerted at that dose and to minimize them as well as to work out a suitable technology for the radiation treatment in the multipurpose industrial-scale irradiation plant called AGRORAD.

1. Material and methods

1.1. Test material

A charge of 1000 class 1 deep-frozen chicken carcasses from one production line, packed in polyethylene bags and in boxes of ten, was investigated.

The carcasses came from one slaughtering plant of the Government owned plant VEB Chicken and Geese Production Mockrehna. The chickens were hybrids of the Cornish and White Rocks species line N 23. The test material was stored on pallets at −18 °C in the cold storage VEB Kühlbetrieb Leipzig.

1.2. Radiation treatment

The deep-frozen chicken carcasses were irradiated in two PANORAMA cobalt-60 plants (nominal activity 0.8 or 0.2 PBq) of the Central Institute of Isotope and Radiation Research, Leipzig, of the Academy of Sciences, at a room temperature of 15 °C.

The dose rate in both plants was about 0.5 kGy h^{-1}, the ratio of D_{max}/D_{min} being at 1.5 or 1.3, respectively. For the determination of the D_{10} value doses of 0.0; 0.1; 0.2; 0.5; 1; 2 and 4 kGy were applied. The doses used for the sensorial investigations were 2.5, 4.0, 6.0 and 8.0 kGy.

An exact dosimetry was carried out in the lower dose range (up to 0.5 kGy) using ferrous sulphate FRICKE dosimeters and by means of chlorobenzene

dosimeters in the higher range. In the multi-purpose industrial-scale irradiation plant of AGROGRAD (type GBS 84) 430 eviscerated chicken were irradiated in two tests at an average dose rate of 0.35 kGy h^{-1} and a D_{max}/D_{min} ratio of 1.5 using isolation containers. The overall dose was 4 kGy (3.3 – 5.0).

1.3. Microbiological investigations

The aim of the investigations was to carry out microbiological examinations on chicken carcasses irradiated at 0.05 – 4 kGy and on unirradiated ones, in order to find the safe irradiation dose for Salmonella decontamination.

After analysing the behaviour of the normal microflora, irradiated and unirradiated carcasses — after contamination with a definite amount of *S. typhimurium* — were examined microbiologically and the D_{10} values for these bacteria as well as for further indicator microorganisms were determined. Table 1 shows the irradiation programme for carcasses, each contaminated with 6×10^7 (test 1), 13×10^3 (test 2), or 12.7×10^5 (test 3) colony forming units, respectively. Subsequent contamination experiments using *S. typhimurium* at levels equal to those expected in intensive poultry breeding and in the production of eviscerated chicken, and examinations of naturally, contaminated chicken carcasses *(S. heidelberg)* were carried out in order to establish the optimum irradiation dose.

In a pilot-scale test described below 10 t of *S. heidelberg*-contaminated chicken carcasses were used to test a dose of 4 kGy for its safety and applicability.

1.4. Sensory tests

The sensory evaluation of unirradiated chicken carcasses and of those irradiated at doses between 2,5 and 8 kGy was carried out in accordance with

Table 1

Irradiation programme for chicken carcasses, artificially contaminated by S. typhimurium

Irradiation programme	Test 1	Test 2	Test 3
S. typh. contamination (cells per carcass)	6×10^7	13×10^3	12.7×10^5
Number of contaminated carcasses	48	36	27
Number of carcasses per group and dose	6	4	3
Irradiation doses (kGy)	0.05; 0.1; 0.2; 0.5; 1.0; 2.0; 4.0; add.: 6.0		add.: 6.0, 8.0
Time between irradiation and microbiological examination (in days)	21–49	30–90	14–40

the guidelines issued by the Office of Standardization, Measurements and Goods Control of the GDR, evaluating three characteristics:

exterior and
interior qualities — weighting factor 1 (number of points counted once)
smell — weighting factor 1
taste — weighting factor 2 (number of points counted twice)

The examination pattern used containing defined test criteria (Table 2a, b, c) was worked out by the Magdeburg Veterinary Hygiene Inspection and by the former Institute of Meat Production Magdeburg. A national standard for poultry meat does not yet exist in the GDR. The pattern used renders the examination results reproducible and it makes possible comparisons of quality which in the case of merely descriptive expert opinions could not be drawn.

Table 2/a

Sensory quality test — Assessment of poultry (farm poultry): Appearance (Internal qualities)
(weighting factor: 1)

Unweighted score	Properties
5 (very good; positive properties are very distinct., no faults or defects)	characteristic of the species, typical colour of stewing meat, pleasantly juicy, without deviations in colour, without escaped protein coagulants, very easy to cut, very tender; fat: yellowish — white (pullet), yellow (adult animals)
4 (good; little faults and defects: food value scarcely affected)	characteristic of the species, typical colour of stewing meat, juicy, only very slight deviation in the shade of colour, few escaped protein coagulants, easy to cut, tender; fat: white — yellowish
3 (still according to standard; some faults and/or deficiencies but not below minimum quality standards)	still characteristic of the species; colour of stewing meat with slight deviations in the shade of colour — even in the form of blemished spots, mediumgrade in terms of juiciness: slightly dry, meat juices escape with protein coagulants, tough consistency; fat: white — greyish
2 (not conforming to standard; faults and/or deficiencies, minimum quality standards not met, appropriate for consumption)	not quite characteristic of the species, typical deviations of colour with the stewing meat, blemished spots, shade of colour not quite according to species, dry, slightly mealy, heavy outflow of meat juice with protein coagulants, very tough; fat: greyish, slightly oily
1 (not to be marketed: eatable after processing: gross faults and deficiencies)	not according to species characteristics: colour of stewing meat with heavy deviations: very dry, very heavy flow of meat juice with coagulants, crumbly, mealy, tough; fat: grey, oily
0 (bad, not suitable for consumption)	completely changed colour, complete decomposition, unappetizing, spoiled, not suitable for consumption

Table 2/b

Sensory quality test — Assessment of poultry (farm poultry): Smell
(weighting factor: 1)

Unweighted score	Properties
5+	characteristic of the species, fully aromatic (savoury), pure, fresh
4	characteristic of the species, not quite fully aromatic, somewhat strongly characteristic of the species
3	still characteristic of the species, weakly aromatic, not quite pure and fresh, insipid, slightly sour and stale; fat: slightly stale and soapy as well as like train-oil, slightly oily
2	not quite characteristic of the species, scarcely aromatic, impure, slightly repulsive, clearly stale; fat: stale, strange, moderately soapy and like train-oil, slightly oily
1	not characteristic of the species, not aromatic: clearly impure; a slightly strange smell (odour); smell of staleness prevails; mouldy and musty; fat: soapy, train-oil-like, oily, moderately rancid
0	strongly repulsive; strong strange odour as of old meat: strongly musty, sticky; fat: rancid

+ Scoring classification see Appearance (Internal qualities)

Table 2/c

Sensory quality test — Assessment of poultry (farm poultry): Taste
(weighting factor 2)

Unweighted score	Properties
5+	characteristic of the species; meat and fat fully aromatic, pure, fresh, tender meat
4	characteristic of the species; meat: aromatic, pure, not quite tender, slightly dry: somewhat strongly characteristic of the species; fat: aromatic, pure, somewhat empty
3	still characteristic of the species; meat: poorly aromatic, not quite pure, heavily species-specific; slightly strange taste (influence of fodder or environment); slightly stale and sour; fat: feebly aromatic, slightly stale, very slightly soapy and tasting of train, slightly oily
2	not quite specific to the species; meat: scarcely aromatic; impure, tough, mealy, slightly sour and astringent strange; taste: stale; fat: moderately soapy and tasting of train and oil, moderately oily, strange
1	not characteristic of the species; meat: not aromatic, clearly impure, too tough, too soft, strongly sour; clearly stale, slightly musty; fat: very stale, soapy, tasting of train, oily, moderately rancid
0	meat: powerfully stale, musty, sticky, strongly strange taste; fat: rancid

+ Scoring classification see Appearance (Internal qualities)

The deep-frozen carcasses were thawed for the examination and inspected during dissection. Chest and leg meats were packed separately in aluminium foil, labelled and within 45–60 minutes cooked at 300 °C. On opening the foil the meat was evaluated and its smell tested, then it was cut up and its taste examined. The results were carefully recorded by the respective examiners.

The sensory examinations of one lot of chicken carcasses irradiated at different dose levels were carried out within a period of five months at four weeks intervals.

2. Results

2.1. Microbiological test results

The Salmonella counts per carcass of 61 naturally contaminated carcasses are shown in Table 3. It demonstrates, that the concentration of Salmonellae in naturally contaminated chicken carcasses is normally below one germ per gram.

The irradiation of eviscerated chicken at 2 and 4 kGy leads to a drastic reduction of the normal microflora of the carcasses. The total viable count after irradiation at 2 kGy lies below 10^4 and at 4 kGy below 10^3 per cm^3 of rinsing fluid from the cavity and from body surface. A complete destruction of micro-organisms, however, can only be expected by treatment with a dose above 10 kGy.

In contrast to this, low doses of 0.05, 0.1 and 0.2 kGy seem to cause an activation of the bacteria and a slight increase in the viable count. Between 0.2 and 2 kGy a gradual reduction of the viable count occurred in dependence on the irradiation dose (Fig. 1).

The sensitivity of the various indicator micro-organisms to ionizing radiation increased in about the following sequence: moulds, streptomyces, aerobic

Table 3

Salmonella concentration in 61 naturally contaminated chicken carcasses

Number of Salmonellae per carcass		Number of carcasses	Salmonellae spp.	
>10	<50	39	36×	*S. heidelberg*
			2×	*S. inbandaka*
			1×	*S. give*
>50	<100	13	10×	*S. heidelberg*
			1×	*S. give*
			2×	S. spp., no differentiation
>100	<500	8	8×	*S. heidelberg*
>500	<1000	1	1×	*S. heidelberg*

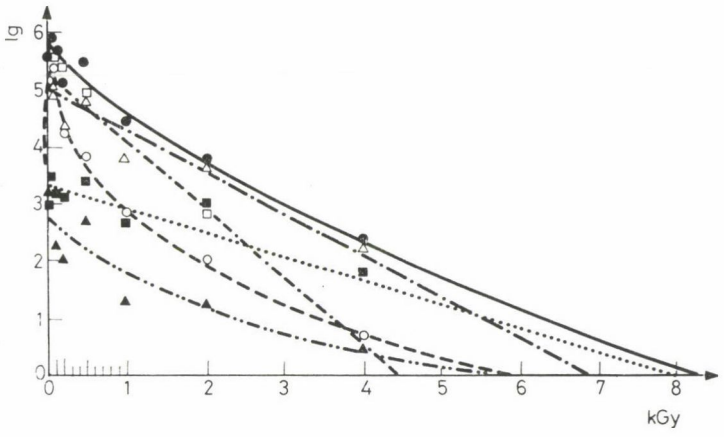


Fig. 1. Comparison of estimated survival curves of total viable count, *S. typhimurium*, Clostridia, aerobic sporeforming micro-organisms, micrococci and lactose-fermenting enteric bacteria in 32 experimentally *S. typhimurium*-contaminated carcasses (6×10^7) bacteria per eviscerated chicken) ●———● total viable count; △———·———△ micrococci; ○-----○ Clostridia; ■·····■ coliform lactose-fermenting enteric bacteria; ▲-·-·-▲ aerobic sporeforming microorganisms; -·-·-·- *S. typhimurium*

sporeforming organisms, yeasts, flavobacteria xanthomonas, micrococci, enteric bacteria and pseudomonads.

In carcasses artificially contaminated by 6×10^7, 1.27×10^6 or 13×10^4 *S. typhimurium* bacteria in the chest and peritoneal cavities, pathogenic organisms in carcasses contaminated by 10^7 bacteria could — after irradiation at an average dose of 4 kGy — only be detected by means of enrichment procedures; in carcasses contaminated by 10^6 and 10^4 germs no detection was pos-

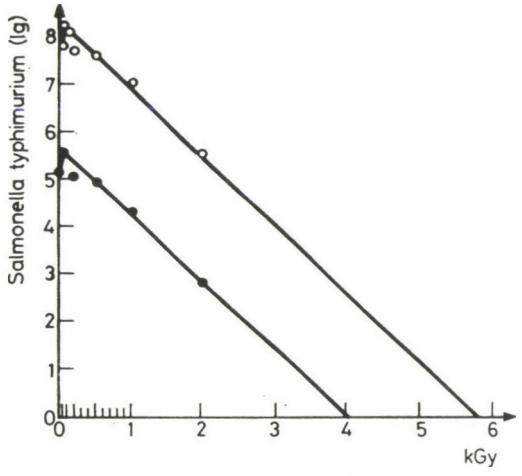


Fig. 2. Concentration of *S. typhimurium* cells in artificially contaminated broiler carcasses (6×10^7 cfu) in dependence on the radiation dose. ○———○: Salmonellae per carcass (500 cm³ rinsing fluid); ●———●: Salmonellae per cm³ rinsing fluid

Table 4

Concentration of S. typhimurium *per cm³ rinsing fluid (500 cm³) of carcasses, each contaminated with* 6×10^7 S. typhimurium *cells, in dependence on irradiation dose*

Irradiation (kGy)	Laboratory 1		log	log x	Laboratory 2		log	log x	log x overall $\bar{x}$	Standard deviation $\pm s$	log x per carcasse (500 cm³) $\bar{x}$
	No carcass	cfu per cm³			No carcass	cfu per cm³					
0	25	6.0×10^5	5.7782								
	26	1.8×10^5	5.2553								
	55	1.5×10^4	4.1761	4.9529	32	2.4×10^5	5.3802				
	56	4.0×10^4	4.6021		31	2.4×10^5	5.3802	5.3802	5.0952	± 0.240	7.7942
0.05	37	4.2×10^5	5.6232								
	38	6.0×10^5	5.7782								
	43	2.0×10^5	5.3010	5.5761	30	3.2×10^5	5.5051				
	44	4.0×10^5	5.6021		29	4.0×10^5	5.6021	5.5536	5.5690	± 0.060	8.2680
0.1	39	5.0×10^5	5.6990								
	40	7.0×10^4	4.8451								
	41	5.0×10^5	5.6990	5.4035	28	3.2×10^5	5.5051				
	42	3.0×10^5	5.4771		27	5.2×10^5	5.7160	5.6106	5.4902	± 0.405	8.1892
0.2	35	5.9×10^5	5.7709								
	36	1.0×10^6	6.0000								
	47	2.0×10^5	5.3010	5.0378	26	1.8×10^5	5.2553				
	50	1.2×10^3	3.0792		25	6.6×10^3	3.8193	4.5373	4.8710	± 1.160	7.5700
0.5	33	1.4×10^4	4.1461								
	34	2.5×10^5	5.9223								
	48	7.0×10^4	4.8451	4.9223	24	4.0×10^4	4.6021				
	49	2.0×10^5	5.3010		23	2.0×10^5	5.3010	4.9516	4.9322	± 0.200	7.6312
1.0	31	3.0×10^4	4.4771								
	32	4.0×10^4	4.6021								
	51	5.0×10^4	4.6990	4.2698	22	2.0×10^4	4.3010				
	52	2.0×10^5	5.3010		21	6.4×10^4	4.8062	4.5537	4.3531	± 0.451	7.0521
2.0	29	3.0×10^2	2.4771								
	30	5.0×10^2	2.6990								
	53	2.0×10^3	2.3010	2.8578	20	1.2×10^3	3.0792				
	54	9.0×10^3	3.9542		19	2.0×10^2	2.3010	2.6901	2.8019	± 0.258	5.5009
4.0	27		<1.6990								
	28		<1.6990								
	45	$<5.0 \times 10^1$	<1.6990	<1.6990	18	$<5.0 \times 10^1$	<1.6990				
	46		<1.6990		17	$<5.0 \times 10^1$	<1.6990	<1.6990	n. d. (enrichment positive)		

n.d.: Exact amount of Salmonellae not detectable, enrichment positive; cfu.: colony forming units

Table 5

Concentration of S. typhimurium *per cm³ rinsing fluid (500 cm³) of carcasses, each contaminated with* 12.7×10^5 S. typhimurium *cells, in dependence on irradiation dose*

Serial number of carcass	Number of carcasses	kGy	cfu	log cfu	log x cfu $\bar{x}$	Standard deviation $\pm s$	log x cfu per carcass (500 cm³) $\bar{x}$
70			76.0×10^2	3.8803			
71	3	0	18.0×10^2	3.2553	3.4790	± 0.349	6.1778
72			2.0×10^3	3.3010			
64			2.5×10^3	3.3979			
65	3	0.05	11.0×10^3	4.0414	3.5801	± 0.402	6.2791
66			2.0×10^3	3.3010			
74			5.0×10^3	3.6990			
75	3	0.1	7.0×10^3	2.8451	3.5147	± 0.599	6.2137
76			10.0×10^3	4.0000			
67			1.8×10^3	3.2553			
68	3	0.2	9.0×10^3	3.9542	3.5845	± 0.351	6.2835
69			3.5×10^3	3.5441			
77			8.0×10^2	2.9031			
78	3	0.5	12.0×10^2	3.0792	2.7459	± 0.434	5.4449
79			1.8×10^2	2.2553			
61			5.0×10^1	1.6990			
62	3	1.0	3.0×10^2	2.4771	2.0071	± 0.414	4.7061
63			7.0×10^1	1.8451			
58–60	3	2,0	$< 5.0 \times 10^1$	n.d.	n.d.	—	n.d.
55–57	3	4.0	0	0	0	—	0

n.d.: Exact amount of Salmonellae not detectable, enrichment positive
cfu.: colony forming units

sible even using multiple fractionated enrichment, which is at present considered the most sensitive method (in the media of Rappaport-Vasiliadis, potassium tetrathionate broth according to Preuss or selenite broth). Tables 4 and 5 show the decrease of colony forming units of *S. typhimurium* per cm³ rinsing fluid of carcasses, each contaminated with 6×10^7 or 12.7×10^5 *S. typhimurium* bacteria, respectively, in dependence of irradiation dose. The resulting inactivation curves are demonstrated in Figs. 2 and 3.

In the case of 10^4 *S. typhimurium* bacteria per carcass, which is about equal to naturally infected flocks of poultry, already an irradiation at 2 kGy brought about a complete Salmonella-inactivation (Fig. 4).

The D_{10} values for *S. typhimurium* could only be calculated for the first two groups of artificially contaminated chicken carcasses and, being 0.74 and 0.57, respectively, are well in agreement with those found in the literature (El-Fouly & Kramomtong, 1980; Mulder, 1982).

The D_{10} value of 0.74 determined by means of the regression function $y = a + bx$ in the case of a carcass artificially contaminated by 6×10^7 *S.*

typhimurium bacteria requires a radiation dose of 5.7 kGy and, after artificial contamination by 1.27×10^6 *S. typhimurium* bacteria and a D_{10} value of 0.57, a dose of about 3.5 kGy for complete Salmonella inactivation, respectively (Figs. 2 and 3).

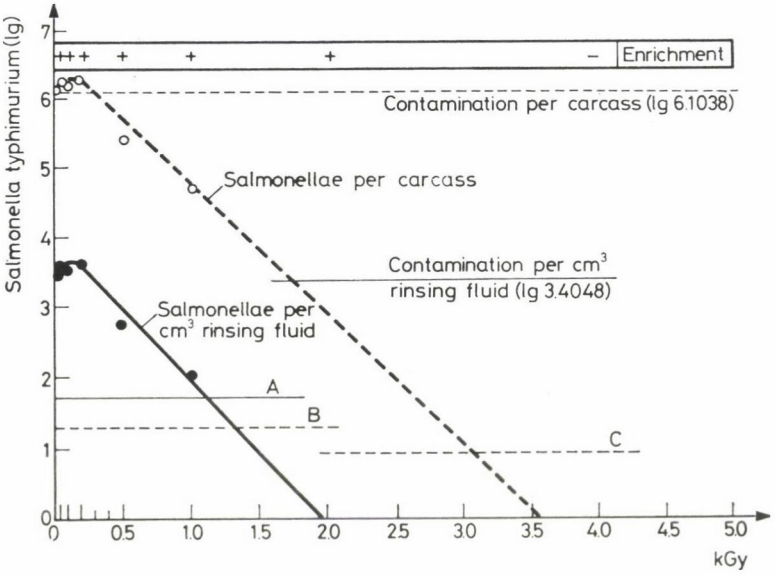


Fig. 3. Concentration of *S. typhimurium cells* in artificially contaminated broiler carcasses (12.7×10^6 cfu) in dependence on the radiation dose. A: Sensitivity of the direct culture method (per cm³ rinsing fluid), B: Sensitivity of the enrichment method (per cm³ rinsing fluid), C: Sensitivity of the enrichment method (per carcass)

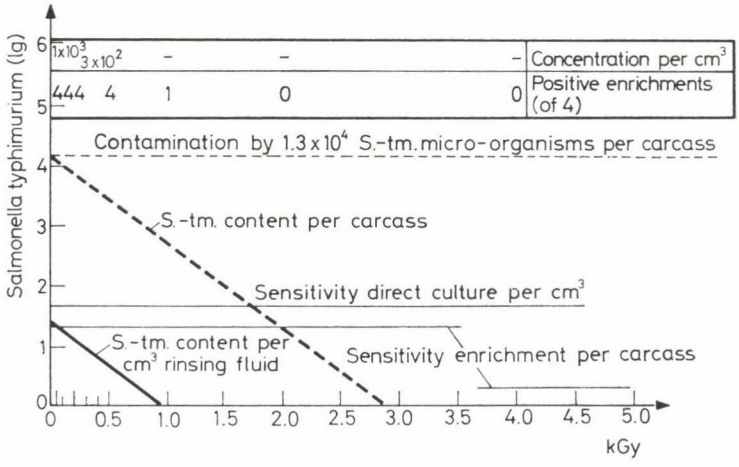


Fig. 4. *S. typhimurium* concentration in eviscerated chicken, contaminated into the body cavity by 1.3×10^4 *S. typhimurium* bacteria per carcass, in dependence on the radiation dose

Table 6

Comparison of frequencies of Salmonella detection in 150 chicken carcasses irradiated at 4 kGy and in 105 unirradiated ones by means of 5-fold fractionated enrichment using three different enrichment media[a]

Salmonella species	Unirradiated carcasses					Irradiated carcasses				
	1	2	3	4	5	1	2	3	4	5
S. heidelberg	4	10	38	50	55					1
S. inbandaka				1	2					
S. give			1	1	2					
S. species non-identified				1	2					
sum total	4	10	39	53	61					1
percent of carcasses examined	3.8	9.5	37.1	50	58.1[b]					0.7[c]

1, 2, 3, 4, 5: enrichment fraction
[a] sum total of the three enrichment procedures
[b] related to 105 chicken carcasses
[c] related to 150 chicken carcasses

Since in practice, with naturally contaminated chicken carcasses, Salmonella concentrations of less than 10^5 Salmonellae per carcass can be expected, for Salmonella inactivation in those a dose of 2.5–4.0 kGy is sufficient.

The radiation treatment of naturally contaminated chicken carcasses *(S. heidelberg)* at 4 kGy, with the expection of one out of 150 carcasses examined (= 0.7%), led to the complete inactivation of Salmonellae. The positive finding occurred in the fifth enrichment fraction of the sodium tetrathionate broth; a smear infection cannot be ruled out. In contrast to the irradiated carcasses, the examination of 105 unirradiated chicken of the same charge yielded 61 times Salmonellae (= 58%), of which already 3.8% occurred in the first enrichment fraction. The results show how widespread Salmonellae are in naturally infected chicken stocks (Table 6).

The irradiation dose of 4 kGy is a safe treatment for chicken carcasses heavily contaminated with Salmonellae which guarantees a complete destruction of all Salmonellae up to a contamination ratio of 10^6–10^7 Salmonellae per carcass.

2.2. Sensory test results

The results show that the sensory characteristics deteriorate with increasing dose and duration of storage.

Eight weeks after irradiation noticeable alteration could only be found in carcasses irradiated with 8 kGy, while after twelve weeks even in cases of 6 and 4 kGy lower numbers of scores points were given. After five months the

Table 7

Comparison of selected sensorial test characteristics of chicken carcasses irradiated at 4 kGy in one of the PANORAMA plants (room temperature: 15 °C) and unirradiated carcasses

Characteristics	Chicken carcasses		Weighting factor
	irradiated	unirradiated	
consistency	3.52	3.49	1
smell	2.92	3.19	1
taste	2.73	2.78	2
overall evaluation	11.90	12.24	

quality of the chicken carcasses did no longer meet taste requirements. The sensory values found in the tests were, however, obtained under the most unfavourable conditions, because the experiments in the PANORAMA irradiation plant of the Central Institute of Isotope and Radiation Research were carried out at a room temperature of 10 °C and cooling could not be applied during irradiation. The low dose rate in the plant made long exposures necessary.

Comparative sensorial examinations of unirradiated and irradiated chicken carcasses after treatment with only 4 kGy in isolation containers yielded no differences in quality (Table 7). As in the preliminary examinations, the most striking deviation for the better can be observed in the characteristic of smell.

3. Pilot-scale test

3.1. The AGRORAD irradiation plant

A pilot test in which 10 t of chicken carcasses were irradiated was to prove that the irradiation treatment procedure can be put into practice.

Aluminium containers used in the AGRORAD plant for product irradiation were for the purpose of isolation lined with a layer of polystyrene and a further coat of aluminium. Subsequent experiments showed temperature differences of 4–6 °K within 24 hours, provided the chicken carcasses in the containers had on leaving the cold storage an interior temperature of −18 to −20°C. Thus the prerequisites had been created for the interior temperature of the deep-frozen chicken carcasses after irradiation and transportation not to exceed −12 °C considerably.

The 10 t of "objectionable" chicken carcasses were a lot from the stock of VEB Mockrehna which, after an acute *S. heidelberg* process, had been rejected with positive findings by veterinary hygienists ten days before slaughtering.

Taking the established D_{10} value of 0.57 as a basis, the Salmonella count could be reduced by 7 or about 4 decimal power by means of radiation treatment at 4 or 2.5 kGy, respectively. The decision about which dose should be applied is thus clearly dependent on the initial microbial load. Since at present information about these figures available in the GDR is not sufficient, the radiation treatment, in the interest of safety, is carried out at an average dose of 4 kGy. A reduction of this to 2.5 kGy can be considered after more experience about the safety of the method and further data on the initial load will have been obtained. This would result in a reduction of cost, an increase in throughput, shorter exposure and, thus, a better command of the cooling regime during irradiation as well as a lower impairment of sensorial properties.

In analogy to the preliminary experiments the pilot-scale test also revealed that the influence on smell after irradiation is clearly changed for the better. Consistency and taste did not show any differences between irradiated and unirradiated chicken carcasses.

The results of the microbiological investigations also confirmed those of the preliminary experiments.

The 10 t of irradiated chicken meat were sold in various forms:

— The manufacturers VEB Mockrehna processed part of the meat to produce poultry "Hamburger".

— 1500 portions of rice boiled in stock containing chicken fricassee were prepared in a Leipzig large-scale canteen.

— The production line at Schildau of the VEB Poultry Industry manufactured the following products:

poultry "liver cheese"	(400 kg)
turkey "breakfast meat"	(200 kg)
broiled sausage "Berliner Art" made of poultry	(1 t) and
turkey "Bierschinken" sausage	(600 kg).

The products were evaluated according to TGL (which is an abbreviation for "Technische Normen, Gütevorschriften und Lieferbedingungen" in the GDR meaning Technical Standards, quality regulations and conditions of delivery) and labelled as marketable.

3.2. Technology, throughput and price of radiation treatment for chicken carcasses in the AGRORAD irradiation plant

From the findings established two possible industrial applications of irradiation technology for poultry carcasses in the GDR follow:

The slaughtering plants themselves can construct an irradiation facility (type GBS 84) where the total output of chicken carcasses can be irradiated.

In the GDR there exist five large-scale poultry slaughtering plants. The output of such a plant working single-shift is about 35 t of carcasses per day. A plant on the principle of GBS 84 would have to be designed for a load of 350 kCi (= 13 PBq) in order to be capable of treating the total output of such a slaughtering plant at an average dose of 4 kGy.

Another possibility is that a selection of chicken carcasses labelled "fit after treatment" are transported to the existing AGRORAD irradiation plant for treatment. That means, only those chicken carcasses are irradiated here, in which Salmonellae were detected in control examinations immediately before slaughter, or which showed heavy Salmonella-contamination at the time of slaughtering.

In the AGRORAD plant the irradiation of the products is performed in positions of maximum dimensions of $1.0 \times 1.2 \times 2.7$ m and maximum capacities of 1 t each (which is correspondent to one double container).

The radiation treatment of chicken carcasses must be carried out under conditions of deep-freezing (i.e. the increase of the interior temperature of the carcasses not exceeding -12 °C) throughout 24 hours. The 10 t pilot test proved that the isolation containers used at present are suitable for the technological realization of the irradiation with regard to dose (4 kGy), overdose factor (1.63), technological throughput and maintenance of the cold chain during transport and irradiation.

With a loading of the plant at about 120 kCi cobalt-60 (4.4 PBq), the realization of a dose of 4 kGy requires a duration of irradiation of 12 hours (cycle time: 90 min). In cases of higher loadings of the plant (up to 300 kCi = 11 PBq) these durations can be shortened according to Table 4. In the same way exposures are clearly shortened, if it appears that only a dose of 2.5 kGy will be required for the radiation treatment of the chicken carcasses.

Two assumptions were made for the mean throughput per hour: the isolation containers used for irradiation had either (like in the 10 t pilot test) a maximum capacity of 250 kg or of 350 kg. But from an economical point of view it is necessary to load at least 550 kg per container (700 kg per position or double container), in order to guarantee optimum throughput and minimum irradiation cost. It is being considered not to pack the polyethylene bags containing the carcasses in boxes of ten but as loose goods into the containers for irradiation.

The development of optimum irradiation containers as well as work on transport, turnover and storage processes will be the next tasks within the framework of further realizing the procedure on an industrial scale.

These technological problems pending and because of the fact that particular health hazards for the population come from objectionable chicken

Table 8

Durations of irradiation and passage of 10 or 16 containers as well as mean throughput at a capacity of 250 or 350 kg of carcasses, respectively, in the industrial scale irradiation plant of AGRORAD in dependence on the cobalt-60 load, calculated for radiation doses of 2.5 and 4.0 kGy

Dose	Duration of irradiation (h)	Cycle time (min)	Duration of passage for a charge of		Cont/d	Cont/d mean throughput	
			10 containers	16 containers		0.25 t per cont.	0.35 t per cont.
			(h)			(kg h^{-1})	
2.5 kGy							
120 kCi	7.5	56	11.25	14	51	533	683
150 kCi	6.0	45	9	11.25	64	667	853
200 kCi	4.5	34	6.25	8.5	99	889	1320
250 kCi	3.6	27.5	5.4	6.75	106	1110	1420
300 kCi	3.0	22.5	4.5	5.6	128	1330	1700
4.0 kGy							
120 kCi	12	90	18	22.5	32	333	426
150 kCi	9.6	72	14.4	18	40	416	533
200 kCi	7.2	54	10.8	13.5	53	555	711
250 kCi	5.8	43	8.6	10.8	66	694	888
300 kCi	4.8	36	7.2	9	80	833	1067
						η: 20%	η: 26.6%

carcasses, we have decided to use the second possibility and, for the time being, to irradiate only those which are labelled "fit after treatment".

At a load of the plant of 120 kCi it is possible to irradiate 8–10 t of chicken carcasses per day at a dose of 4 kGy. The price would be at 800 M (Marks GDR), if about 350 kg could be loaded per container.

In judging the capacity of the plant it should be taken into consideration that during September/October the plant is used for the irradiation of onions and other agricultural produce. For chicken carcasses a mean capacity of 4–5 t per day is available during the other months.

3.3. Benefit/cost ratio for the irradiation of different quantities of chicken carcasses per annum

On the level of the manufacturers of the chicken carcasses there are economic losses in the case of slaughtered chicken being blocked from processing, since carcasses labelled "fit after treatment" will be degraded from class I to class III. Thus the price of 7308 M/t for class I is reduced to 5394 M/t for class III, which implies losses of 1914 M/t.

In the case of a capacity for the irradiation of 1000 t of chicken carcasses per annum, the irradiation cost is about 900 M/t, including transport, cooling, etc.

From these figures an annual net profit increase of about 1 million M results for the manufacturers.

The power consumption per ton of preserves is expected to be 2.7 to 3.3 MWh. In contrast to this, the power used up by irradiation is negligible, so that 3000 MWh can annually be saved.

The value of the goods improved by the procedure, which are thus preserved as meat for grilling, is about 7 million M.

Chicken carcasses labelled "fit after treatment" amount to 2500 t per year in the GDR. In order to be able to irradiate these in AGRORAD, an additional 80 kCi (2.96 PGq) cobalt-60 will be required .

The irradiation cost for such amount of 2500 t per year, calculated per ton including transport, cooling, etc. amounts to about 675 M.

Annually avoided losses for the manufacturers (i.e. net profit increase) in this case will be at about 3 million M. The value of the improved goods production is about 18 million M.

The main gain from the introduction of the irradiation procedure is the contribution to avoid health risks for the population. The economic losses by Salmonella-induced intestinal diseases can be demonstrated in the following example:

According to the Statistical Yearbook of the GDR (STATISTISCHES JAHRBUCH 1986) 10 717 cases of salmonellosis caused by foodstuffs occurred in 1984. Assuming, in accordance with investigations carried out in Canada and the USA that 10% of them are due to contaminated poultry, about 1000 of the above cases can be related to poultry. If in 96% of the cases a mild course of the disease is assumed and 4% are severe cases implying admissing to hospital, at least 250 000 M will be required for treatment.

From the data for industrial production and the number of factory and office workers given in the Statistical Yearbook, an average output of more than 500 M per day and worker can be calculated. Assuming losses of one working day for a mild effect and ten days in a severe case, deficits of 680 000 M in industrial production will arise. Thus at least 900 000 M of losses would result for the economy from salmonelloses caused by poultry carcasses only. Certainly all the initial parameters for the calculation will be in practice considerably higher and potential deaths have not been taken into consideration at all.

For the irradiation of 2500 t of chicken carcasses per year 1.25–1.75 million M will be required.

For the production of chicken carcasses, on the other hand, a net profit of 4.75 million M can be realized due to avoiding reduction in quality (benefit/cost ratio 2.7–3.8).

In the USA a benefit/cost ratio of 2.2–4.2 was calculated for the irradiation of chicken carcasses.

From the economic point of view, apart from preserving the eviscerated chicken as meat for grilling (not expressed in terms of money here), there will be substantial savings in power that would be required for processing the carcasses as preserves, namely 7500 MWh worth 2.5 million M. By raising the processing capacity, the duration of cold storage can be shortened, which implies further savings of 750 000 M.

The cost for the treatment of salmonelloses and production losses in working hours of at least 900 000 M can also be saved, so that an overall gain of not less than 4.15 million M will be achieved.

4. Conclusions

The irradiation dose of 4 kGy is a safe treatment for chicken carcasses heavily contaminated with Salmonellae, which guarantees a complete destruction of all Salmonellae up to a contamination rate of 10^6–10^7 Salmonellae per carcass.

Comparative sensorial examinations of unirradiated and irradiated chicken carcasses after treatment with up to 4 kGy yielded no differences in quality.

Production manufactured by using the irradiated chicken meat were in accordance to valid quality standards in the GDR. These production were sold in various forms:

- speciality "Hamburger"
- 1500 portions of rice boiled in stock containing chicken fricassee
- poultry "liver cheese" (400 kg)
- turkey breakfast meat (200 kg)
- broiled sausage "Berliner Art" made of poultry (1 t) and
- turkey "Bierschinken" sausage (600 kg).

Calculations of throughput and price of radiation treatment for chicken carcasses in the AGRORAD irradiation plant result in benefit/cost ratio of 2.7—3.8.

*

The research project rendered possible by the kind assistance of the Government owned plant Chicken and Geese Production Mockrehna and the cold storage VEB Kühlbetrieb Leipzig and VEB Kühlbetrieb Halle. Our thanks are due to the staff of the AGRORAD irradiation plant.

Literature

DAHLGREEN, R. R., ISRAELSON, B. L., MARSHALL, D. B., MIRENTI, R. A., REUTZEL, L. F., RONNING, D. C. & TROUP, C. M. (1982): Irradiation of sterilized chicken meat: A feeding study in rats. Raltech Scientific Services. St. Louis. *Natl. Techn. Inf. Serv.* PB. 84–187038.

EL-FOULY, M. Y. & KRAMOMTONG, I. (1980): Elimination of pathogenic organisms from chicken carcasses by irradiation. — Part II. Radiation survival of Salmonella nilose on artificially contaminated chicken. International Facility for Food Irradiation Technology, Wageningen, Netherlands, *IFFIT Report* No. 2. pp. 1–16.

MCGOWN, E. L., LEWIS, C. M., ROBLES, A., WARING, P. P., SKOLA, J. H., GILDENGOVIN, V. L. & SAUBERLICH, H. E. (1981): Investigations of possible antivitamin B_6 properties in irradiation sterilized chicken. Div. Res. Support. Letterman Army Institute of Research, Inst. Rep. No. 87, Presidio of San Francisco, Calif. *Natl. Techn. Inf. Serv.*, PB 84–187079.

MORRISON, R. M. & ROBERTS, T. (1985): Food irradiation: New perspectives on a controversial technology. A review of technical, public health and economic considerations. *Natl. Techn. Inf. Serv.*, PB 86–177474.

MULDER, R. W. A. W. (1982): *Salmonella radiation of poultry carcasses.* Wageningen. Doctoral thesis, Landbouwhogeschool, The Netherlands.

WIERBICKI, E. & JOSEPHSON, E. S. (1986): Ionizing radiation energy used in food preservation and pest control. *Ass. int. ind. Irrad. News., 16*, 64–119.

STATISTISCHES JAHRBUCH (1986): *Statistisches Jahrbuch der Deutschen Demokratischen Republik.* Staatsverlag der Deutschen Demokratischen Republik, Berlin. p. 381.

Acta Alimentaria, Vol. 19 (2), pp. 125—137 (1990)

INVESTIGATION OF THE STATE OF WATER IN FIBROUS FOODSTUFFS BY NEAR INFRARED SPECTROSCOPY

K. J. KAFFKA, L. HORVÁTH, F. KULCSÁR and M. VÁRADI

Central Food Research Institute, H-1022 Budapest, Herman Ottó u. 15. Hungary

(Received: 1 August 1988; revision received: 8 September 1989; accepted: 12 September 1989)

In order to find out more about the physical properties of free water and water bound by different energies we studied the optical properties of distilled water, paprika flesh, paprika seeds and powdered paprika in the 1100–2500 nm near infrared wavelength region.

Our studies were performed on a 6450 type NEOTEC (Pacific Scientific) Research Composition Analyzer (RCA). The data analysis and evaluation were performed on a NOVA III computer attached to the RCA. With these instruments and technique we were able to study the bounding and structure of water incorporated in foodstuffs.

The different paprika samples were dried in three different ways: in a drying oven, in a microwave oven and in a calcium carbide desiccator. We recorded the spectra during drying and thus we were able to draw conclusions about the evaporating materials from the spectral differences.

Our experiments with distilled water proved the Iwamoto mixture model according to which the absorption peaks of water found in the near infrared spectrum are composed of the spectra of three different species of water molecules.

The Iwamoto model was proved at an absorption peak around 1400 nm. He established that the spectrum peak of water is the resultant of the peaks of three different species of water molecules, namely S_0 at 1418 nm; S_1 at 1466 nm and S_2 at 1510 nm.

In our Institute we proved this at around 1900 nm for water. According to our findings a water molecule S_0 has a peak at 1908 nm, whereas S_1 at 1932 nm and S_2 at 1974 nm, respectively.

We noticed that during drying the different water species evaporated in a different way as a result of the method used. In the case of using a drying oven — heat being transferred externally — or using calcium carbide, water molecules of S_0 type leave the material first while in the case of applying a microwave oven — heat being transferred internally — due to their dielectric properties, water molecules of S_1 and S_2 type leave the material either together with or even prior to S_0 type water molecules. In the case of external heat transfer a number of S_1 and S_2 type water molecules obviously firstly become S_0 type water molecules and leave the material like that. The shift of the peak of the resultant spectrum during drying was remarkable.

No absorption peak shifts were noticed during rehydration which means that the rehydration process is not reversible; only water molecules of S_0 type take part in it.

Keywords: near infrared spectroscopy, analysis of foods, moisture

Better understanding of bounding and structure of water has been an interesting topic for researchers in the food industry for a long time. Water is an important constituent of almost any food and at the same time the life

element of useful and harmful microorganisms. The knowledge about water is of prime importance at food preservation in reducing energy consumption of dryers and evaporators.

NIR and NIT techniques have opened new fields for finding out more about how water is incorporated in foods and also the structure of water.

The 1100–2500 nm wavelength region is near to visible electromagnetic radiation. We can find characteristic energy absorption peaks for the majority of the different constituents of foods in this region. The absorption frequency is characteristic for the structure of the molecule whereas the magnitude can refer to its concentration. This was the basis for establishing rapid non-destructive composition analysis using NIR/NIT technique for the different constituents of food such as water, proteins, fats, fibers, starch, carbohydrates, sugar, acids, alcohol, etc. Composition analyzers based on NIR/NIT technique consist of two main parts: a special high speed spectro-photometer and a computer connected to it. The spectrum measured by the spectro-photometer is the resultant of the spectra of all the constituents therefore the complicated relationship between the concentration of one constituent and the characteristic details of its spectrum are deciphered by the computer.

When we used an RCA of a higher output and greater sensitivity it was noticed that there were differences among the spectra of freewater and water bound by different energies in foods. At the same time researchers noticed another phenomenon, too; the spectrum of water is highly dependent on temperature and the shift of spectrum caused by the change of temperature could not theoretically be explained.

Török (1955) studied the structure of water already in 1955 in his thesis. Our knowledge concerning water was significantly increased by the information Szalai (1964) provided us with in his thesis in 1964.

In the past few years a number of researchers used NIR/NIT technique — a quick non-destructive method — for determining the moisture content of raw and dried paprika. The first results of the combined research performed at the Central Food Research Institute and the Research Institute for the Canning Industry, were published by Horváth and Nádai in 1981. Further results were published by Püspök and Horváth and Horváth and Püspök in 1983. After these promising results the Central Food Research Institute decided to perform a more detailed physical analysis of water contained in ground paprika.

The main topic of Iwamoto's (1987) lecture at the International NIR/NIT Conference (Budapest, 1986) was the shift of the absorption peaks of free and bound water at different temperatures. Iwamoto (1987) explained this so far not explored phenomenon saying there were three different water species which transfigurate within 10^{-12} s; at one given temperature the ratio of these three species is constant and there is a statistical equilibrium. When temperature changes this ratio changes as well.

The aim of our research was to further study the structure of water and its incorporation in foods. By learning more about the physical properties of water (the three different water species) we can find out more about what happens during drying and evaporation and these findings will contribute to optimizing the energy consumption of production. The moisture content of the product influences its quality and storability thus its quick and exact determination is significant not only from the point of view of energetics. The producer, the processor, the distributor and last but not least the consumer are all interested in this.

1. Materials and methods

We used paprika (red pepper) grown in the Carpathian basin and distilled water in our experiments. We separately studied paprika flesh and paprika seed and powdered paprika from Szeged and Kalocsa.

We prepared sample groups of different paprika flesh and seed chopped to a size of 1 mm. We studied paprika powder (different sorts) in commercially available form, too. These sample groups were dried by different methods and we studied their optical properties (spectra) during these processes. One of the methods was in accordance with the Hungarian Standard MSZ 9681/3—77 at 95 °C in a drying oven; the second method was in a calcium carbide desiccator, whereas for the third method we applied microwave oven for drying.

The samples dried in the drying oven or in the calcium carbid desiccator were in an open glass cup and cooled to room temperature in a covered cup; after this spectra were measured in a standard measuring cell. Thus drying and measuring was performed in two different cups. When drying was performed in a microwave oven this was not possible because of the rapid drying process; in this case drying and measuring both took place in the very same cup.

For measuring reflectance or transflectance spectra a NEOTEC 6450 type Research Composition Analyzer (RCA) was used. Reflectance measurement was performed in a standard cup with a planeparallel glass plate. The diameter of the perpendicularly incident beam was 20 mm. We used a liquid cell of 0.5 mm thickness to measure transflectance whereby the incident beam was reflected by a ceramic diffuse reflector after the beam had passed through the liquid and as a result of this reflectance it passed through the liquid repeatedly before reaching the detector. In both cases we measured 50 spectra in the 1100–2500 nm wavelength range and we used and stored their averages for processing and evaluation. Evaluation was performed on the NOVA III coupled with the RCA. Spectral values were calculated as a logarithm of the ratio of the fluxes reaching the detector from a "white standard" and the sample at an angle of 45°.

In order to study the process of drying we measured the reflectance spectra during all three drying methods and we also studied changes while the samples were rehydrated.

2. Results

Knowing the spectra of the substances released from the sample during drying contributes to evaluating the drying process itself, i.e. identfying the absorption peaks. A significant amount of water, volatile oils and other unstable substances pass from the sample during the process of drying, consequently we also measured the transflectance spectra of distilled water and oil pressed from paprika seed.

In Fig. 1 it can be seen that both water and oil have an absorption band around 1400 nm whereas around 1900 nm only water has an absorption band from which the absorption bands of oil around 1700 and 2150 nm can well be distinguished. For studying the properties of water bound by different energies in paprika the wavelength region around 1900 nm received priority.

When we form the difference between two spectra measured at two different phases of drying we get a differential spectrum representing the spectra of the substances released from the sample. By identifying the peaks of differential spectra we can draw conclusions about the evaporating substances.

Below – as we endeavour to study the influence of the water evaporated during drying upon the spectrum – the differential spectra were measured in the 1800–2200 nm wavelength range. We determined the differential spectra for different paprika samples dried under different circumstances compared to the original spectrum (before drying).

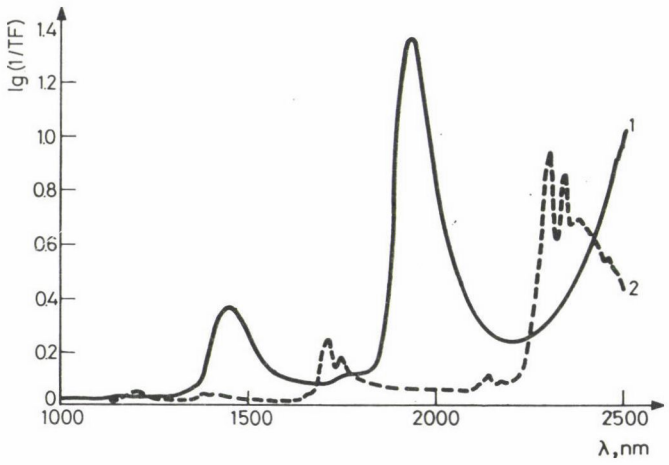


Fig. 1. The diffuse transflectance spectrum of distilled water and volatile oil derived from paprika seed in the 1000–2500 nm wavelength range. 1: Distilled water; 2: Vegetable oil

The formation of the differential spectra for paprika flesh pieces and paprika seed dried in a drying oven at 95 °C can be seen in Fig. 2. and Fig. 3. The drying time is indicated on the curves. The shift of the absorption peak of water towards shorter wavelength is significant in the case of paprika seed.

The differential spectra for paprika flesh pieces and paprika seed dried in a calcium carbide desicator can be seen in Fig. 4. and Fig. 5.

The drying time is indicated on the curves. The shift of the absorption peak for water towards shorter wavelength is again significant in the case of paprika seed.

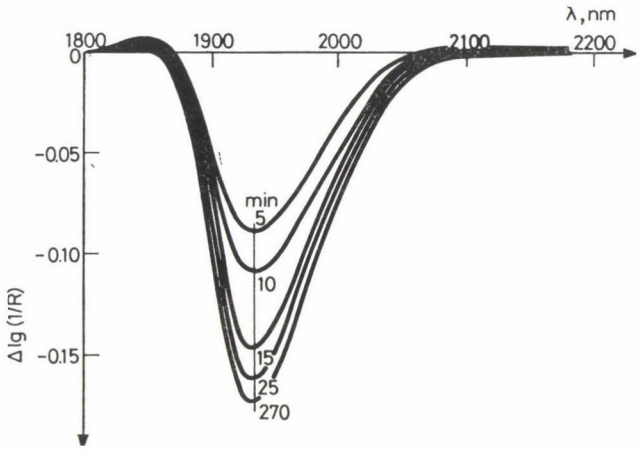


Fig. 2. The diffuse reflectance differential spectra of 1 mm³ pieces of paprika flesh dried in a drying oven at 95 °C — applying different drying times — compared to the initial state in 1800–2200 nm wavelength range

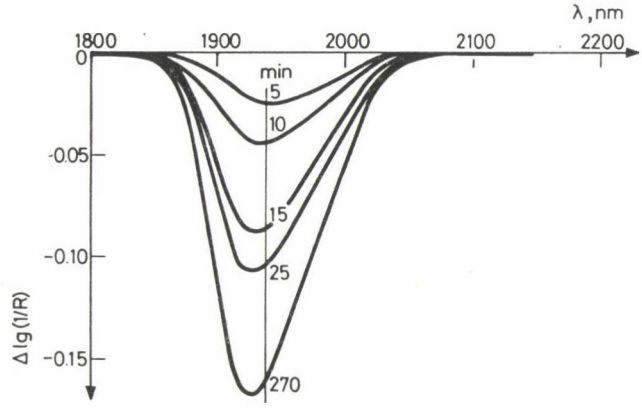


Fig. 3. The diffuse reflectance differential spectra of paprika seeds dried in a drying oven at 95 °C — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

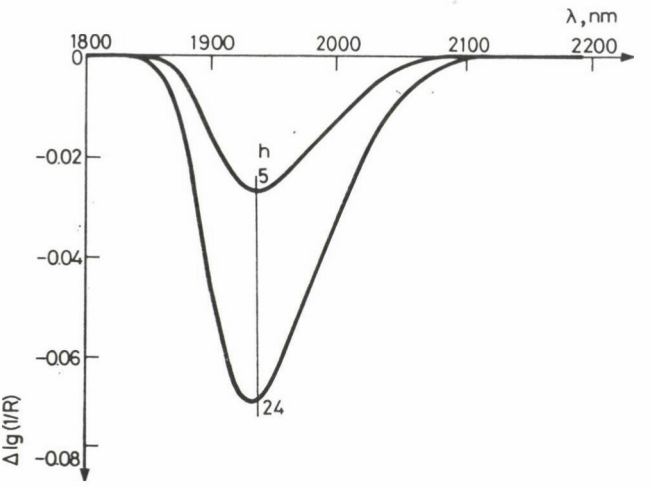


Fig. 4. The diffuse reflectance differential spectra of 1 mm³ pieces of paprika flesh dried in a calcium carbide desiccator in closed air space — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

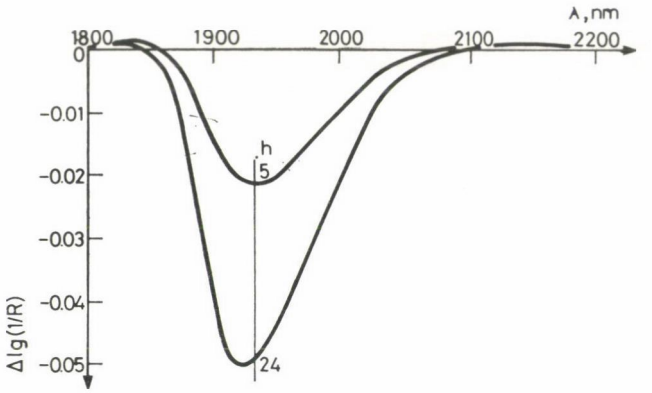


Fig. 5. The diffuse reflectance differential spectra of paprika seeds dried in a calcium carbide desiccator in closed air space — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

The differential spectra for paprika flesh and paprika seed dried in a microwave oven can be seen in Fig. 6 and Fig 7.

The drying time is indicated on these curves, too. Although the character of the differential spectra differ from those of the other two drying methods the shift of the absorption peak of water towards a shorter wavelength is again more significant in case of paprika seed.

The differential peaks for paprika powder from Szeged dried in a microwave oven can be seen in Fig. 8.

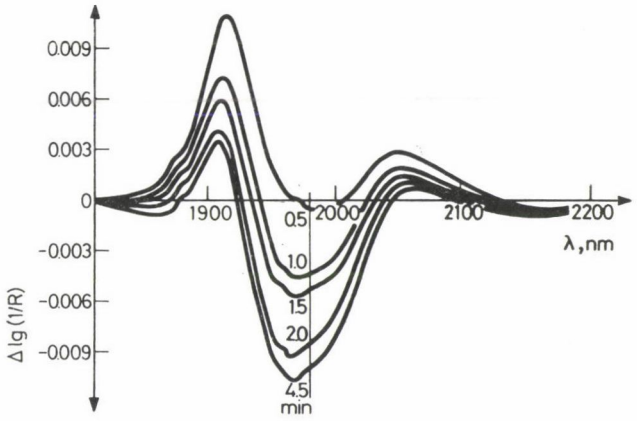


Fig. 6. The diffuse reflectance differential spectra of 1 mm³ pieces of paprika flesh dried in a microwave oven — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

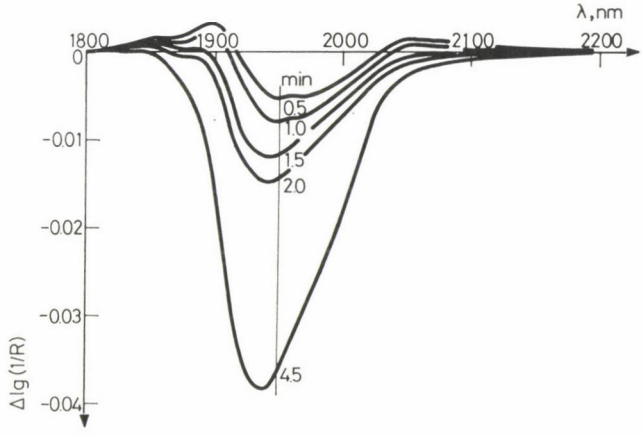


Fig. 7. The diffuse reflectance differential spectra of paprika seed dried in a microwave oven — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

On the spectra the duration of the microwave treatment was indicated. These curves well fit between those in Fig. 6 and Fig. 7; this derives from the fact that paprika powder is a mixture of powdered seed and paprika flesh.

The differential spectra of paprika flesh and paprika seed dried at 95 °C for 270 min and rehydrated at 55% relative humidity can be seen in Fig. 9 and Fig. 10.

The duration of rehydration was indicated on the spectra. No shift was noticed for the absorption peaks of water during rehydratation.

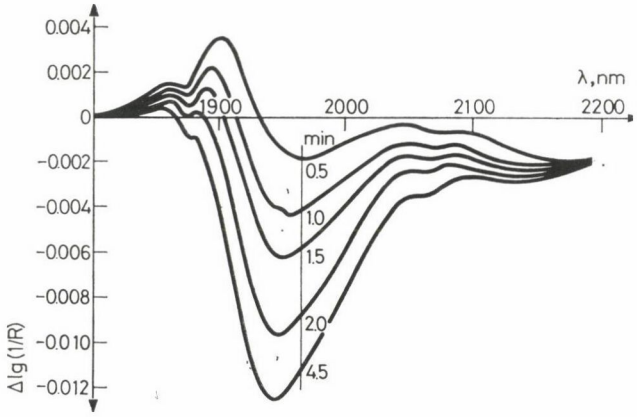


Fig. 8. The diffuse reflectance differential spectra of paprika powder (Szegedi édes nemes brand) dried in a microwave oven — applying different drying times — compared to the initial state in the 1800–2200 nm wavelength range

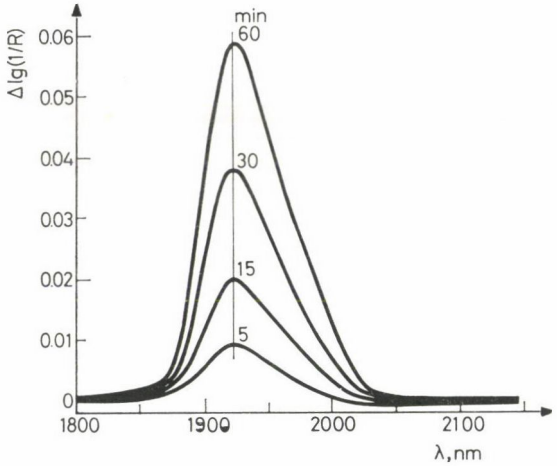


Fig. 9. The diffuse reflectance differential spectra of 1 mm³ pieces of paprika flesh dried in a drying oven at 95 °C (drying time 4.5 hours) and then rehydrated at 25 °C in an air space of 55% humidity

The formation of the log (1/T) spectrum of distilled water and its second derivative can be seen as a function of temperature (Iwamoto measurements) in Fig. 11.

The log (1/T) spectrum of water markedly shifts towards shorter wavelengths as a result of higher temperature whereas the amplitude of the absorption peak of water remains practically unchanged. In contrast to this the second derivative of the log (1/T) spectrum of water possesses three local peaks; these do not shift on the wavelength scale as a result of high temperature but their amplitude changes.

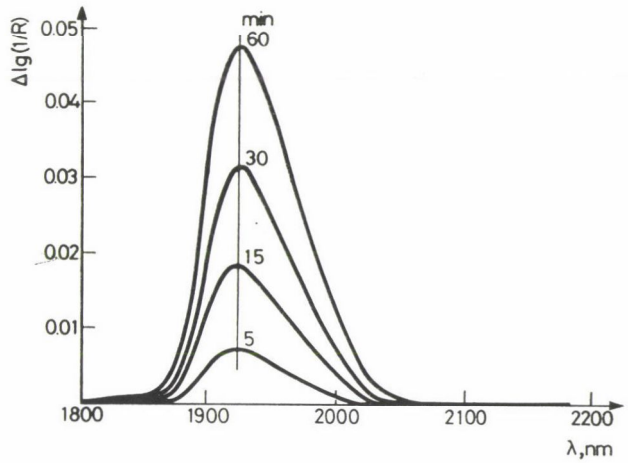


Fig. 10. The diffuse reflectance differential spectra of paprika seeds dried in a drying oven at 95 °C (drying time 4.5 hours) and then rehydrated at 25 °C in ain air space of 55% humidity

Figure 12 shows the spectrum of the second derivative taken with a 10 nm gap of distilled water. This second derivative was transformed from a transflectance spectrum triangularly smoothed with an 8 nm segment (taking into consideration 5 measured points) with a 7.2 nm bandwidth. In the second derivative of the spectrum of water three separate peaks can well be observed at the absorption peak near 1400 nm and 1900 nm. The spectrum in the wavelength range above 2000 nm seems noisy.

3. Conclusions

Paprika is a complicated capillary porous colloid system in which free water can also be found besides water bound by different energies. The water molecules bound with different energies have different resonance frequencies and these are well demonstrated in the diffuse reflection spectra taken in the course of drying.

During the evaluation of the results it must be taken into consideration that in the case of drying in a microwave oven or in a drying oven drying is a result of a heat treatment whereas in the case of calcium carbide desiccation drying happened at room temperature in a physico-chemical way. In the drying oven heat treatment came from the outside; in the microwave oven from the inside. The calcium carbide method is very slow (24 h), the drying oven method takes several hours and drying in a microwave oven is a very rapid method and only takes a few minutes. Beside this we must take into considera-

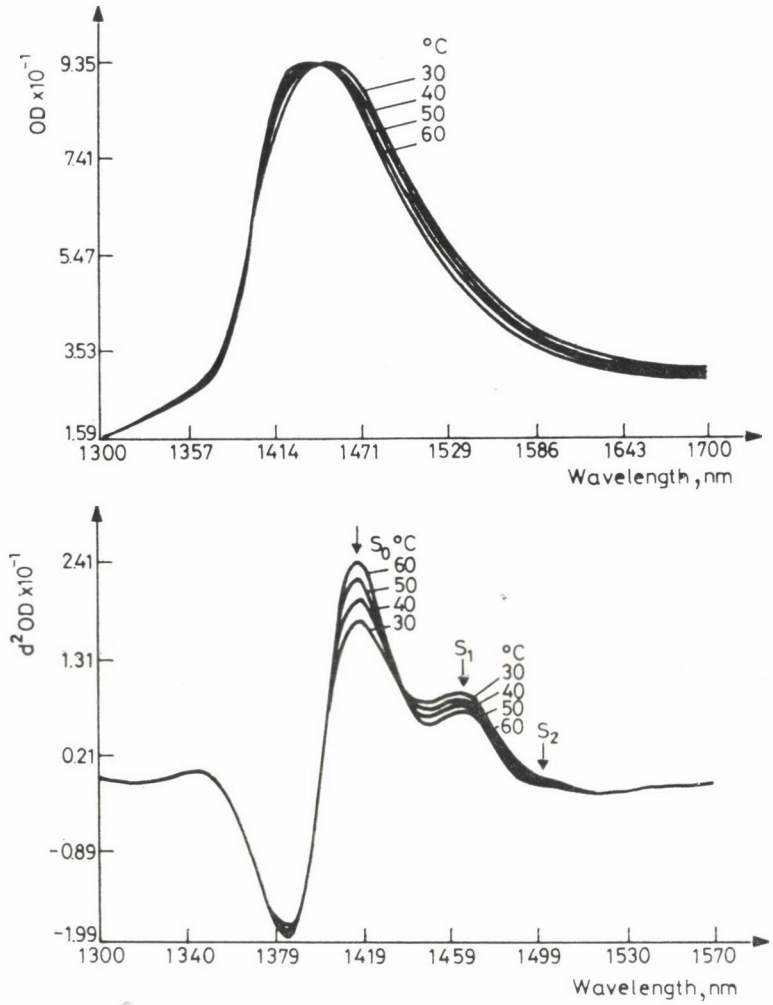


Fig. 11. The log(1/T) spectra of distilled water and their second derivatives measured by Iwamoto at 30, 40, 50 and 60 °C in the 1300–1700 nm wavelength range (International NIR/NIT Conference, Budapest, 1986)

tion that in the microwave oven drying happened in the measuring cell itself covered with a glass window.

As it is well known, in the near infrared wavelength range the absorption peaks of spectra are associated with overtones and combination bands arising from the fundamental vibration bands, and are assigned mainly to C—H, N—H, O—H and C—O bonds in compounds.

In the case of water the O—H bonds are important. The Iwamoto mixture model can be considered proved: accordingly water is composed of different molecular species such as free water molecules (S_0) molecules with one O—H

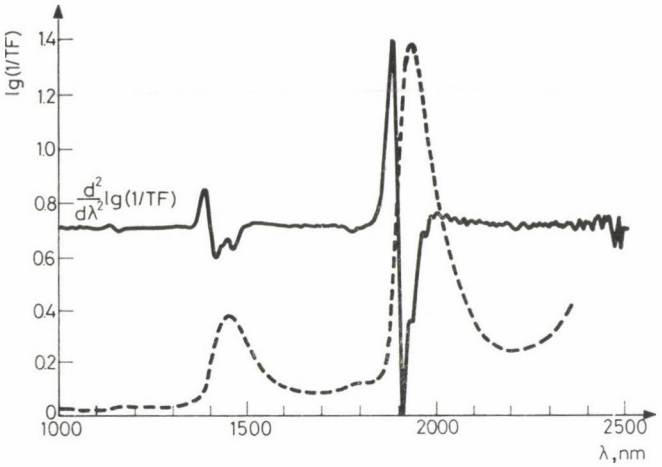


Fig. 12. The log(1/TF) spectra of distilled water with its second derivative measured in KÉKI (Central Food Research Institute, Budapest) at 25 °C in the 1000–2500 nm wavelength range. Spectral bandpass: 7 nm. Smothing: 8 nm triangular. 2nd derivative computed with a gap of 10 nm between points

engaged in hydrogen bonds (S_1) and molecules with two O—H engaged in hydrogen bonds (S_2). Water molecules are in random motion making and breaking these hydrogen bonds at a speed of 10^{-12} s; at a given temperature water consists of an equilibrated mixture of these three molecular species with different numbers of hydrogen bonds per molecule. If temperature changes this ratio changes, too. The evidence to this can be seen in Fig. 11. On the upper part of this figure we can see that the resultant of the log (1/T) spectra of the three molecules shows a leftward shift if the temperature of distilled water is raised from 30 °C to 60 °C in 10 °C steps; meanwhile the amplitude remains unchaged. On the lower part of the figure the second derivatives of the log (1/T) spectra can be found. These spectra show three absorption peaks at 1418, 1466 and 1510 nm wavelength, respectively. These peaks do not shift as a result of changing temperature only their amplitudes change. As a result of raising temperature the amplitude corresponding to S_0 at 1418 nm increases and those of S_1 and S_2 decrease. This clearly indicates that the rate of S_0 molecules increases in case of heating and this is an explanation to the leftward shift of log (1/T) spectra. This phenomenon is in accordance with the theory of thermodynamics which says that higher temperature helps the breaking of O—H bonds.

Iwamoto (1982) performed his experiments with water around the 1400 nm absorption band to avoid the disturbing effects of overtones coming from trace elements. In our experiments measurements were carried out in the absorption band around 1900 nm because we wanted to avoid the disturbing effects of the absorption peaks of volatile oils in paprika around 1400 nm absorption bands. The presence of the three different species of water can well be

demonstrated in this wavelength region, too. The second derivative of log (1/TF) spectrum of water has three peaks in Fig. 12 at 1908 nm, 1932 nm and 1974 nm wavelengths, respectively and these can be attributed to S_0, S_1 and S_2 water molecule species. In Figs. 2–8. it can well be seen that while drying paprika flesh and paprika seed in three different ways absorption peaks show a 15–25 nm shift towards shorter wavelengths. This can be explained with the "mixture" theory (Iwamoto model) as during drying the ratio of the three different species of water changes and obviously first free water (S_0) is released and the water bound by different energies are released only thereafter. In the case of paprika seeds peaks shift more significantly than in the case of paprika flesh during all three types of drying which can be attributed to the difference in composition between paprika flesh and paprika seed, namely, to the higher volatile oil content of paprika seed.

In connection with Figs. 2–3 there is another phenomenon worth noting. The initial rapid drying is followed by a slower and then again a more rapid period before assymptotically reaching the final state. These changes in the speed of drying can univocally followed by standard methods of determining moisture. The explanation to this is that the first rapid change is caused by releasing free water and in the following phases water bound by different energies used up the energy required for their release. The curves in Figs. 6–7 show a different picture, namely in these cases S_1 and S_2 type molecules are immediately released — even as if a little bit earlier than S_0 type molecules. In this case, however, we must know that the dissipation of heat (the extent of heating) is in connection with the dipolmomenta of molecules (dielectric properties). In the case of drying in a microwave oven we must also take into consideration beyond this that the sample was placed in a closed measuring cell and consequently the rapid release of water — steam — could only take place slowly and this also altered the picture of the spectrum. There are still some questions to clear related to the results of microwave drying as drying was performed and spectra were taken in the sample measuring cup itself. Figures 9–10 demonstrate the process of rehydration. It is important to note that during rehydration no shift in peaks can be experienced, which means that while samples again take up water from the air of 55% humidity no changes happen — at least at the beginning — in the ratio of the three water species S_0, S_1 and S_2.

Studying the second derivative of the spectrum of distilled water in longer wavelenth range than 2000 nm the spectrum curves were found noisy (Fig. 12). The presumption was obvious as for better resolution we used very small slits and consequently small energies. Comparing the spectra taken by Karl. H. Norris in the Instrumentation Laboratory of USDA BARC in Beltsville (which Karl H. Norris kindly permitted Károly J. Kaffka to use) to the ones taken here we were surprised at finding these noises being identical and reproducible. As noise is a statistical phenomenon thus irreproducible we

came to the conclusion that the noise — or what we supposed to be noise — was caused by the absorption properties of gases dissolved in water.

It is doubtless that during our studies — though a lot of problems have been cleared — quite a number of questions have arisen and these are still to be answered. It seems that the deeper we penetrate into knowledge — even in the case of such a simple molecule as water — the more details make it more difficult to clarify the relations of nature and the rules of food physics.

Literature

HORVÁTH, L. & NÁDAI, B. (1981): *Részjelentés az Objektív minősítés c. feladat teljesítéséről.* (Report on the accomplishment of the programme on "Objective qualification".) Central Food Research Institute, Budapest, pp. 1–52.

HORVÁTH, L. & PÜSPÖK, J. (1983): Nyers fűszerpaprika nedvességtartalmának meghatározása NIR technikával. "*A NIR technika mezőgazdasági és élelmiszeripari alkalmazása*" c. MTA Tudományos Ülésszak előadásainak összefoglalója. (Determination of the moisture content of raw paprika by NIR technique. Scientific Meeting of the Hungarian Academy of Sciences on the application of NIR technique in the agricultural and food industry. Abstracts.) Hungarian Academy of Sciences, Budapest, pp. 21–22.

IWAMOTO, M. (1987): Preliminary investigation of the state of water in foods by NIR spectroscopy. *International Near Infrared Diffuse Reflectance/Transmittance Spectroscopy Conference.* Akadémiai Kiadó, Budapest, pp. 3–12.

PÜSPÖK, J. & HORVÁTH, L. (1983): Fűszerpaprika őrlemény nedvességtartalmának meghatározása NIR technikával. "*A NIR technika mezőgazdasági és élelmiszeripari alkalmazása*" c. MTA Tudományos Ülésszak előadásainak összefoglalója. (Determination of the moisture content of ground paprika by NIR technique. Scientific Meeting of the Hungarian Academy of Sciences on the application of NIR technique in the agricultural and food industry. Abstracts.) pp. 23–24.

SZALAI, L. (1964): Az élelmiszerek hidratúrájának néhány kérdése. (Some questions of water activity in foods.) Thesis, Budapest.

TÖRÖK, G. (1955): A víz szerepe élelmiszereinkben. (The role of water in our foods.) Thesis of Doctor of Chemical Science, Budapest.

Acta Alimentaria, Vol. 19 (2), pp. 139—155 (1990)

NEW PHASE DIAGRAM OF THE D-GLUCOSE WATER SYSTEM

A. SMELÍK[a], SZ. TÖRÖK[b] and K. VUKOV[b]

[a] Vysoka Škola Technická, CS 81237, Bratislava, Jánska 1. Czechoslovakia
[b] University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

(Received: 28 February 1989; accepted: 12 September 1989)

Saturated D-glucose solutions, depending on temperature, may be in equilibrium with the following solid phases: β-D-glucopyranose, α,β-D-glucopyranose hemihydrate, anhydrous α-D-glucopyranose, β-D-glucopyranose monohydrate, simultaneously present α-D-glucopyranose monohydrate and β-D-glucopyranose monohydrate, α-D-glucoparanose monohydrate.

The solubility curves, presented analytically and graphically, are indispensable in the production of high-purity allotrophe-identical crystalline glucose by means of crystallization at the appropriate temperature and concentration region. The formation and properties of α,β-D-glucopyranose hemihydrate crystals were also demonstrated.

Keywords: crystallization of glucose, glucose solubility, glucose anomers

Pure D-glucose is an important industrial product, a basic raw material of both the food industries and the chemical industries. Strict requirements on purity are specified for glucose used as infusion solution and as a basis material of some syntheses. Crystallization is based on the phase diagram showing the dependence of solubility on temperature and concentration.

Solubility measurements of α-D-glucopyranose were published by JACKSON and SILSBEE (1922) and GILLIS (1923). The changes of D-glucopyranose allotropes as a function of temperature and concentration were presented by NEWKIRK (1924a–f, 1936a, b) in a phase diagram. Based on this the rules of exact control of industrial crystallization were laid down. The reflexion point at 50 °C in the phase diagram of Newkirk marks the formation of α-D-glucopyranose monohydrate. The formation of anhydrous α-D-glucopyranose can be reckoned with, on the one hand, in the supersaturated region of non-stable crystal nucleation between 28 °C and 50 °C. On the other hand, in the supersaturated region of stable crystal formation above 50 °C up to the temperature of $\alpha \rightleftharpoons \beta$ anomer change the described phase diagrams of D-glucopyranose in water were conformed and complemented by YOUNG (1957).

Based on the experiments carried out in the last 18 years by SMELÍK on the solubility and crystallization of glucose YOUNG's diagram (1957) requires modification. This modification has been carried out and the formation of crystalline allotropes hitherto not described has been proven.

Table 1

Phase diagram according to YOUNG of the D-glucopyranose — water system

Equations describing solubility / w — concentration % m/m, ϑ — temperature, °C

Solid phase: ice

$(w-100) = \vartheta\ 11.38\ w - 0.0907\ w^2 - 0.00345\ w^3 - 0.00002653\ w^4$
Range of validity: $-30 < \vartheta < 0$ °C $0 < w < 70\%$

Solid phase: α-D-glucopyranose monohydrate

$w = 33.78 + 0.6215\ \vartheta + 0.00308\ \vartheta^2 - 0.0000231\vartheta^3$
Range of validity: $-4.93 < \vartheta < -54.7$ °C
$30.79 < w < 73.22\%$
Range of stable nucleation: −4.93 °C .... 50 °C
Range of unstable nucleation: 50 .... 54.7 °C

Solid phase: anhydrous α-D-glucopyranose

$w = 53.80 + 0.335\ \vartheta + 0.000365\ \vartheta^2$
Range of validity: $-120.6 < \vartheta < -54.7$ °C
$49.81 < w < 73.2\%$
Range of unstable nucleation: −12.06 .... 50 °C
Range of stable nucleation: 50 .... 54.71 °C

Solid phase: anhydrous β-D-glucopyranose

$w = 67.00 + 0.224\ \vartheta$
Range of validity: $-21.0 < \vartheta < -50$ °C
$62.3 < w < 78.2\%$

1. Materials and methods

In carrying out experiments on solubility the following have to be kept in mind:

— It is a basic condition to keep to a minimum (to a negligible minimum) the error caused by contamination of the reagents used. Therefore crystalline D-glucopyranose of pharmaceutical purity is dissolved, free of other anomers and allotrope-identical.

— Thesis of SMELÍK (1971) changes of pH have a fundamental effect on the solubility of sugars in water. Changes in pH affect the formation of hydrogen bridges. Thus, it is desirable to work with distilled water free of carbon dioxide.

— Antecedent life of the solution (NÝVLT, 1967). To account for this the initial glucose solution should be unsaturated and of a temperature that ensures the structure modifying effect of molecular hydration.

The experimental equipment used ensured reversible phase equilibrium e.g. sealed glass ampoules (Fig. 1). The rate of setting in the reversible phase equilibrium is determined by the total interphase surface. Thus, smaller

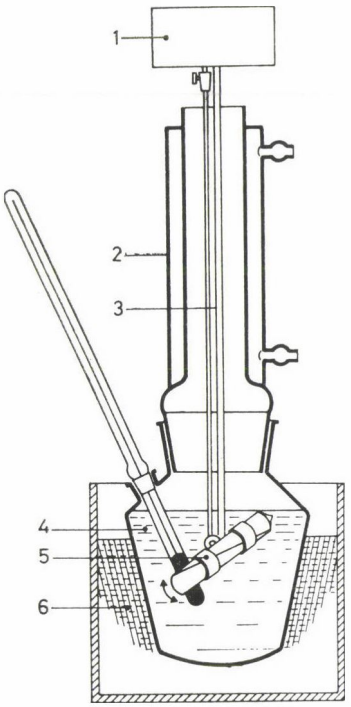


Fig. 1. Equipment to determine the solubility in water of D-glucopyranose. Principle: superfluous amount of D-glucopyranose is mixed with the solution in a sealed ampoule. The ampoule is shaken at constant temperature (in a boiling hot bath). 1: Electro-motor with eccentric outlet; 2: cooler with inner opening permitting the shaking of the ampoule; 3: stainless steel holder; 4: flask with calibrated thermometer; 5: sealed ampule containing the sample; 6: electrically heated container filled with filing

crystals having large surface, are dissolved. The phase equilibrium of the heterogeneous system changes with temperature. Thus the temperature has to be stabilized at a standard deviation of ± 0.05 °C (e.g. by refluxing a boiling liquid). An alcohol–water mixture for example, boils at 95 °C while a glycerin–water mixture at 137 °C.

The optimal contact between phases is ensured by the even distribution of concentration in the whole volume of the suspension. That renders necessary the regulated mixing of the sample (by shaking) which on the other hand ensures the rinsing of the surface of each rolling crystal with the solution. A calculated excess of about 50% of the solid phase does not cause boundary-solvated saturation when added to the unsaturated solution. Solvated saturation occurs if the solid phase is added to a saturated solution.

The increase of concentration manifests itself in the iso-thermal and iso-bar dissolution of the material by well measurable physico-chemical parameters, such as refractive index, density, electric conductivity and other changes.

The table constructed by SMELÍK and co-workers (1968) shows that the stabilization of the solids content of glucose solution, determined by refractometry, can be established with a reproducibility of $\pm 0.05\%$.

Table 2

Time needed to saturate water with D-glucopyranose
(SMELÍK, 1971)

Composition of bath	Temperature, ϑ (°C)	Time (h)	Concentration, w (%)	Solubility (%)		
				Measured (w_n)	Calculated (w_p)	Difference
Ethylalcohol — water	68.4±0.05	0	70.0			
		4	75.66			
		5	76.27	77.44	77.53	+0.09
		6	76.91			
		7				
Ethylalcohol — water	75.85±0.10	0	75.0			
		3	78.58	79.93	79.91	−0.02
		4	79.25			
Ethylalcohol — water	86,25±0,10	0	80.80			
		2	83.63	83.63	83.53	−0.10
		3	83.60			
		4				
Ethylalcohol — water	89.1±0.05	0	79.00	84.47	84.50	+0.03
		2	84.19			
		3				
Ethylalcohol — water	95.5±0.1	0	85.0	86.42	86.56	+0.14
		2	86.8			
		3				
Glycerol — water	100.2	0	85.0	87.92	87.97	+0.05
		2	87.43			
		3				
Glycerol — water	105.5	0	85.0	87.92	87.97	+0.04
		2	87.43			
		3				
Glycerol — water	108.45	0	88.0	90.37	90.25	−0.12
		2	90.04			
		3				
Glycerol — water	109.75±0.05	0	88.5	90.57	90.58	+0.01
		2	90.41			
		3				
Glycerol — water	111.25±0.05	0	89.0	91.11	90.96	−0.05
		2	90.89			
		3				
Glycerol — water	114.8	0	90.0			
		1	91.66	91.95	91.82	−0.13
		2	91.91			
		2.5				
Glycerol — water	121.1±0.05	0	91.5	92.50	92.61	+0.11
		1	92.57			
		1.5				

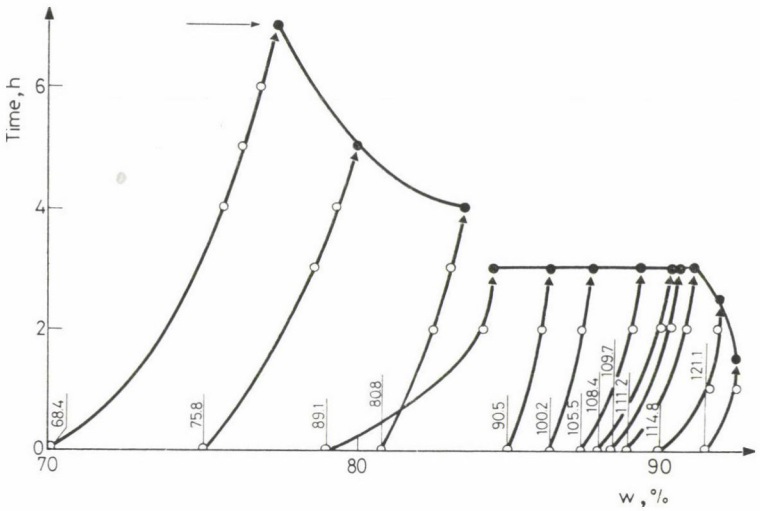


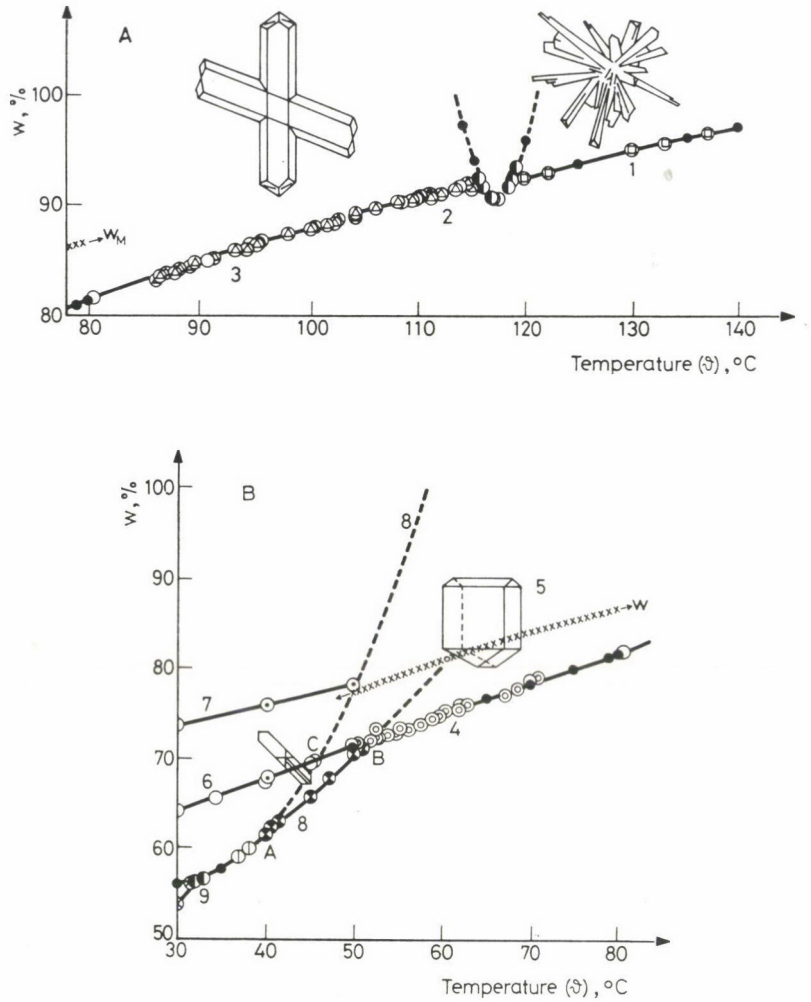
Fig. 2. Change in the concentration (w) of D-glucose solutions as a function of temperature (ϑ) and saturation period (τ): w — f(ϑ . τ) Abscissa: D-glucose concentration, w, %; ordinate: saturation period, h; dry matter content of the saturated solution

The time-dependence of the saturation of water with saccharose was studied by HRUBÝ (1937, 1938), GRUT (1937, 1938) and CHARLES (1959, 1960). According to data in Table 2 the saturation of water with D-glucopyranose accelerates with increasing temperature. At a temperature of 70 °C saturation takes about 7–8 h. Above this temperature it gradually accelerates and upon reaching 80 °C it becomes stable and takes 3 h. The shortening of the saturation period is observable again in the temperature range of 115–120 °C. As revealed by Fig. 2 the relationship to saturation time and temperature is not regular and continuous. Some independent and characteristic phases of saturation are observable the physico-chemical explanation of which may be traced back to changes in the allotropies of D-glucopyranose.

2. Results

The phase diagram of the D-glucopyranose – water system is presented in Fig. 3 based on the data obtained by the above described methodology and reliable references in the literature. The characterization of individual phases as well as the curves plotted in the points of measurement, are described in short. The figure shows the characteristic crystal formations of glucose in each phase.

Individual sections of the phase diagram in decreasing order of temperatures (ϑ) are as follows:



For legends and text see p. 145

Region of stable crystallization of β-D-glucopyranose. Temperature limits $118.4 < \vartheta < 155.7$ °C and the corresponding concentration limits $91.36 < w < 100.0\%$.

$$w_\beta = 31.30 + 0.734\,\vartheta - 0.00188\,\vartheta^2. \quad (1)$$

The accord of the points with the curve is within $\pm 0.12\%$ limits. The upper limit of the curve is the melting point of anhydrous β-D-glucose.

Saturation section of the labile anomer transformation. In this region the α and β anomer are in labile equilibrium in the solution. The temperature limits of the region are close: 113.4 and 121.0 °C.

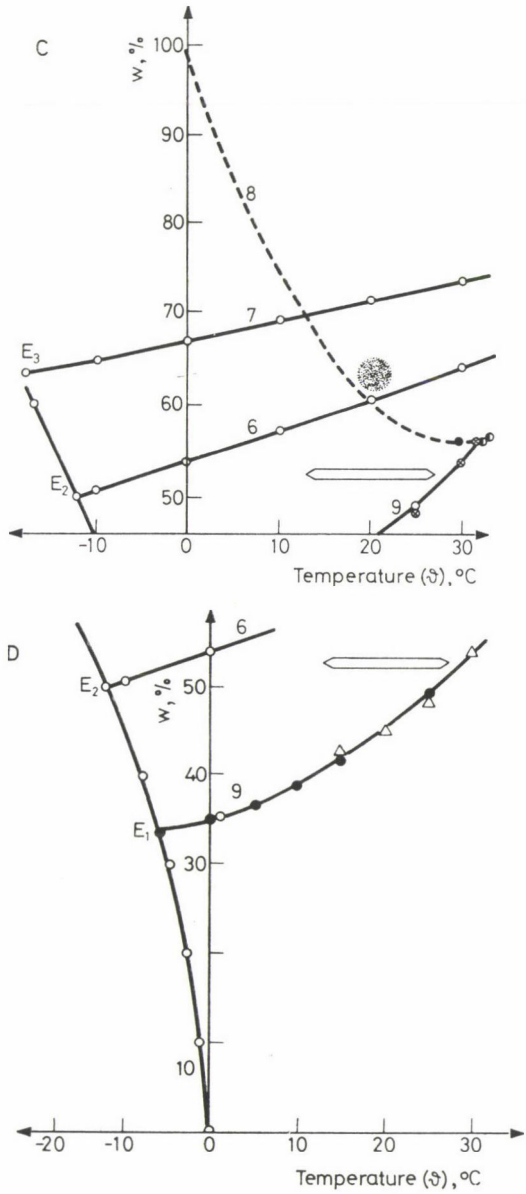


Fig. 3. Improved phase diagram of the D-glucopyranose — water system. Abscissa: temperature, (ϑ) °C; ordinate concentration of D-glucose, w% (m/m); the crystals formed are represented by schematic drawings. For printing technical reasons broken into 4 sections (A, B, C, D). Section A: 1-orthorombic β-D-glucose, eq. (1); 2 — labile region of anomer transformation, eq. (2); 3 — orthorombic α,β-D-glucose hemihydrate, eq. (3); Section B: 4 — rhombic bisphenoidale anhydrous α — D-glucose, eq.(4); 5 — probable limit of metastable supersaturation, eq. (7); 6 — unstable region of α-D-glucose saturation, eq. (5); 7 —unstable region of β-D-glucose saturation. eq.(6); 8 — composite monocline crystals of α-D-glucose monohydrate and β-D-glucose monohydrate, eq.(8); 9 — labile region of anomer transformation; section C: 6, 7, 8: see section B; 9 — monocline α-D-glucose monohydrate; section D: 9 — see section C; 10 — solid phase: ice

The curve calculated on the basis of the experimental points of SMELÍK (1971) approximating within $\pm 0.10\%$ margin of error is a parabola, determined by the following equation:

$$w_{\alpha \rightleftharpoons \beta} = 8614.2 - 145.46\ \vartheta - 0.6205\ \vartheta^2. \quad (2)$$

Solubility has a definite minimum here at 117.2 °C.

Crystallization region of dimer α,β-D-glucopyranose hemihydrate. The crystalline phase consists in columns formed from α and β anomers bound through a water bridge containing orthorombic elongated, mostly twin crystals. The temperature limits of the region are 79.1 and 115.8 °C and the limits of concentration 80.95 and 92.06%. The solubility curve within $\pm 0.08\%$ margin of error is expressed by the following equation:

$$w_{\alpha, \beta} = 39.575 + 0.6744\ \vartheta - 0.00191\ \vartheta^2. \quad (3)$$

Crystallization region of anhydrous α-D-glucopyranose. This falls in the temperature range of $51.6 < \vartheta < 79.1$ °C and in the concentration range of $71.74 < w < 80.95\%$ of the saturated solution. The equation of the solubility curve with margin of error $\pm 0.11\%$ approximation limiting stable nucleation according to SMELÍK (1971):

$$w_{\alpha, s} = 51.08 + 0.4433\ \vartheta - 0.00083\ \vartheta^2. \quad (4)$$

A further section of the saturation curve between 51.6 and −12.1 °C (E2) is aproximated by YOUNG (1957) with the equation:

$$w_{\alpha, u} = 53.80 + 0.335\vartheta + 0.000365\vartheta^2. \quad (5)$$

This forms the lower limit of the unstable crystallization of anhydrous α-D-glucose. The upper limit also given by YOUNG (1957) is β-D-glucose saturation curve.

$$w_{\beta, u} = 67.00 + 0.224\vartheta \quad (6)$$

with validity limits + 50.0 °C and −21.0 °C eutectic point (E_3) being the continuation of the latter saturation curve in the stable crystallization region of anhydrous α-D-glucose (between 51.6 °C — 79.1 °C) (Fig. 3) describing according to SMELÍK (1961) the curve of the metastable saturation curve:

$$w_m = 55.63 + 0.523\vartheta - 0.017\vartheta^2. \quad (7)$$

Meaning of indices:

α and β corresponding anomers,
s: stable,
u: unstable,
m: metastable.

The crystallizing anhydrous glucose form rhombic bisphenoid crystals.

Crystallization region of β-D-glucopyranose monohydrate. In this region polymorphous β-D-glucopyranose crystals may be formed. This region extends over the concentrations above the stable saturation curve between temperature limits $39.8 < \vartheta < 51.2$ °C (Fig. 3, area bordered by points A, B, C). It continues in the metastable region up to temperature 60.4 °C where it has a common point with metastable supersaturated α-D-glucopyranose solutions.

Mixed crystallization region of α-D-glucopyranose monohydrate and β-D-glucopyranose monohydrate. In this temperature region D-glucose monohydrate is a labile modification. The temperature limits are $32.3 < \vartheta < 39.8$ °C, the limits of concentration $56.23 < w < 61–63\%$. In this region the transitional points on the solubility curve of α-D-glucopyranose are at temperatures 39.8 °C and 32.3 °C. The joint saturation curve of the two modifications (α and β-D-glucose monohydrate) continues over the unstable area towards both the higher and lower temperatures.

The curve is of parabola shape, its minimum being at 29.05 °C, the accuracy of approximation in the saturation region is $\pm 0.05\%$. Its equation:

$$w_{\alpha \rightleftharpoons \beta\ H_2O} = 98.638 - 2.9557\ \vartheta + 0.05087\ \vartheta^2. \quad (8)$$

The extreme values of the curve up to 100% solubility are between −0.46 °C and + 58.5 °C.

Crystallization region of α-D-glucopyranose monohydrate. In this region glucose crystallization in the form of monocline monohydrate crystalls. The crystallization by stable nucleation of α-D-glucopyranose starts at the inflexion point at 32.3 °C temperature and continues to the eutectic point of −5.6 °C temperature (E_1). The approximating equation of the saturation curve (within $\pm 0.28\%$ limit of error):

$$w_{\beta,\ H_2O} = 35.19 + 0.2355\ \vartheta + 0.01291\ \vartheta^2. \quad (9)$$

Conclusions

3.1. Crystallization of β-D-glucopyranose

Anhydrous β-D-glucopyranose crystals are formed in the solution when the number of D-glucose molecules is higher than that of the water molecules. This is theoretically valid up to the melting point of D-glucose (155.67 °C). (In practice, for known reasons, the melting point is between 148 and 155 °C.)

The crystallization of β-D-glucopyranose has no practical significance. The crystals formed are spherically oriented small needles. The viscosity and stickiness of the mother liquor practically prevents separation of the crystals. When washed with water the anhydrous glucose becomes hydrated and mutarotation occurs (a mixture of α-D-glucopyranose and β-D-glucopyranose is formed).

United with hot absolute pyridine an anhydrous β-D-glucopyranose-pyridine additive compound is formed which, as a result of appropriate treatment liberates pyridine and thus pure, anhydrous β-D-glucopyranose is obtained, identifiable by infrared spectroscopy (SMELÍK, 1975).

3.2. Crystallization from allotrope-labile saturated solutions

It has been known for a long time that in glucose solutions of high temperature racemization occurs and α-D-glucopyranose and β-D-glucopyranose are formed simultaneously. In the phase diagram given here this appears in the close temperature region between 115.8 °C and 118.4 °C. The solubility curve reveals a minimum at 117.19 °C where the concentration of the saturated solution amounts to 90.91 mass %.

3.3. Crystallization of α,β-D-glucopyranose dimer hemihydrate

In the temperature range of 115.8 °C and 79.1 °C in supersaturated glucose solutions anomer hybrid dimer hemihydrate crystals are formed. The supersaturated solution is prepared from a D-glucose solution of 88–89% by raising its temperature to 110–120 °C. When the solution is then cooled to 90 °C well developed dimer hemihydrate crystals are formed. These can be separated from the solution by raising the temperature for a short period to 95 °C. The crystals washed in glacial acetic acid grow in idiomorph orthorhombic form (Fig. 4). Analyzed by X-ray diffraction the dimer crystals differ from α-D--glucopyranose and β-D-glucopyranose crystals.

The absolute melting point of the dimer crystals is twofold: 145.97 ± 1.33 °C and 158.2 ± 2.7 °C. This is the consequence of melting in parallel of the α-anomer and β-anomer in the racemized crystal lattice.

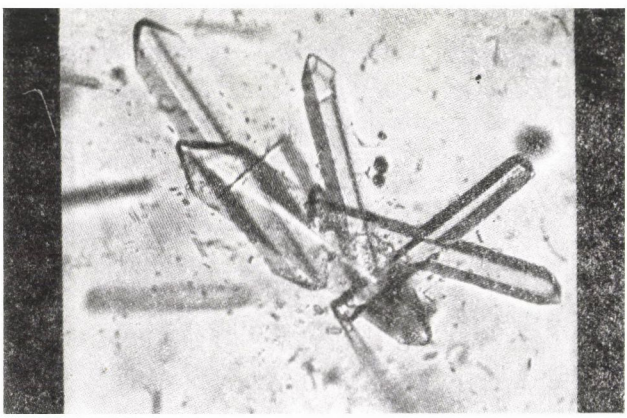
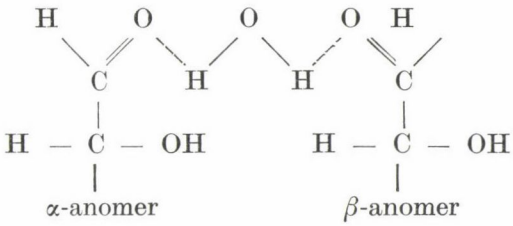


Fig. 4. Orthorhombic idiomorph crystals of α, β-D-glucopyranose hemihydrate. Microphoto: 27.6-fold enlargement with MEOPTA microphoto fitting on 65 × 90 mm, and MO-1 orthochromatic plate, exposition 35 s

On the basis of optical rotation directly upon dissolution of the crystals it is assumed that between the α-anomer and β-anomer a water molecule serves as a bridge, thus developing the elements of the racemic hemihydrate crystal:



This bridge is stable, up to 140 ± 1 °C temperature no siginificant loss of mass occurs and the samples do not melt.

3.4. Crystallization of anhydrous α-D-glucopyranose

In the temperature range between 79 1 °C and 51.6 °C anhydrous α-D-glucopyranose separates from the supersaturated solution in the form of rhombic bis-phenoid crystals.

Below 60.4 °C temperature in the above temperature range, in the case of unstable nucleation, the crystallization of α-D-glucopyranose monohydrate may occur. Therefore, to crystallize anhydrous α-D-glucopyranose temperatures between 60–65 °C are suggested (NEWKIRK, 1924b, 1936b).

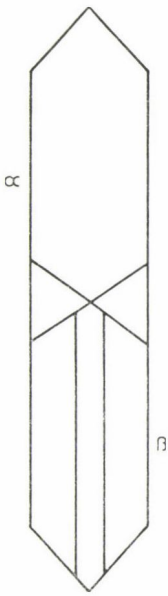


Fig. 5. Idealized drawing of the composite crystal of α-D-glucopyranose(α)monohydrate and β-D-glucopyranose (β) monohydrate (DEAN, 1974)

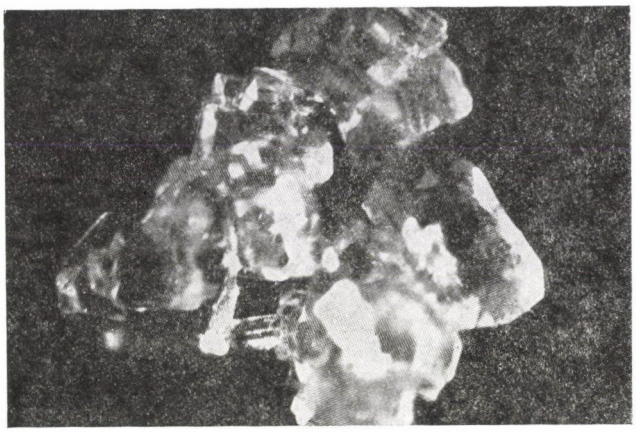


Fig. 6. Polymorph crystals in the unstable nucleation range of α-D-glucopyranose. 150-fold enlargement

In the course of this experiments SMELÍK (1971) succeeded to separate from an aqueous glucose solution of 83 $\pm 1\%$ density and an initial temperature of 90 °C slowly decreased to 64.5 ± 1 °C under mild mixing pure anhydrous crystals and prevented them from recrystallization with simultaneous water binding by washing in alcohol. The rhombic bisphenoid character of the crystals can be seen in Fig. 5. The crystallization range of such anhydrous α-D-glucopyranose crystals is very narrow. The step-wise growth characteristics of molecular crystals is observable (in manyfold enlargement) in Fig. 6.

The crystals obtained in accordance with the above recognitions were analyzed by infrared spectrography, X-ray diffraction and differential thermoanalysis and the results prove unambiguously that these crystals represent pure anhydrous α-anomers.

3.5. Crystallization of β-D-glucopyranose monohydrate

HOLESZOVÁ (1967) observed that when further insoluble crystalline anhydrous α-D-glucopyranose was transferred into saturated solutions then a dehydrating effect arose and in consequence the solution became highly supersaturated. That seems to show that the supersaturation of solutions is possible by dehydration (Table 3, Fig. 7).

RYBÁROVÁ (1975) developed a method for the determination of "true" solubility. By this method α-D-glucopyranose monohydrate was obtained in pure configuration. The solubility curve determined by the author has inflexion points at 39.85 °C and 32.26 °C. The solubility curve comes to an end at the theoretically calculated eutectic point (E_1) at -5.6 °C temperature and 33.47% concentration. JACKSON and SILSBEE (1922) place the above mentioned cryohydrate point to –5.3 °C temperature and 31.75% concentration of the satur-

Table 3

Dehydrating effect of anhydrous α-D-glucopyranose when added to the saturated solution (HOLESZOVÁ, 1967)

Temperature ϑ (°C)	Pseudo saturation w (%)	Dehydrating supersaturation w (%)
13.2	42.11	47.87
16.0	45.39	48.87
19.8	46.92	50.92
25.0	50.62	54.93
35.0	57.55	61.14
45 0	65.51	67.16
50.1	71.18	71.84

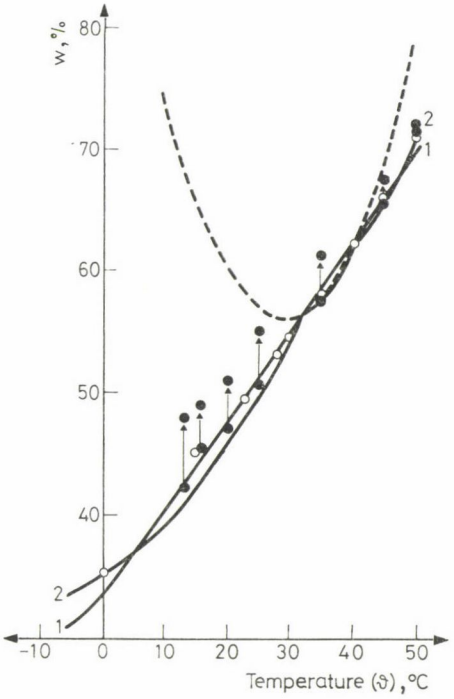


Fig. 7. Pseudo-saturation and true solubility as observed in the crystallization region of D-glucopyranose monohydrate. Abscissa: temperature (ϑ) °C, orginate: concentration, (w)%. Symbols: 1: anhydrous α-D-glucopyranose pseudo-saturation according to the calculation of YOUNG (1957); 2: true saturation with α-D-glucopyranose monohydrate based on the experiments and calculations of SMELÍK (1980); ○: experimentally established pseudo-solubility (JACKSON & SILSBEE, 1922; CILLIS, 1923). ● experimentally established pseudo-solubility with the possibility of supersaturation (HOLESZOVÁ, 1967)

ated solution while YOUNG (1957) found the following values —4.95 °C and 30.79%.

In the crystallization experiments of DEAN (1973, 1974) α-D-glucopyranose monohydrate and β-D-glucopyranose monohydrate resulted in complex crystals permitting of the conclusion that this is the growth range of stable β-D-glucopyranose monohydrate. This assumed region is shown in the new modified phase diagram (Fig. 3) by the triangle marked A, B, C. This is situated above the actual saturation limit between the temperatures of 39.85 °C and 51.19 °C or up to 60.39 °C, thus, forming the nucleation section between the stable and unstable β-D-glucopyranose monohydrate.

The temperature of 39.85 °C determines the inflexion point in the solubility curve. The temperature of 51.19 °C is common with the saturation curve of anhydrous α-D-glucopyranose and 60.39 °C is a common point with the metastable supersaturated glucose solution.

3.6. Labile crystallization of α-D-glucopyranose monohydrate and β-D-glucopyranose monohydrate configurations

NEWKIRK (1923, 1924a—f, 1929, 1930a, b, 1936a, b, 1939) as well as EBERT and co-workers (1927a, b) require solutions of as low viscosity as possible for the technical crystallization of D-glucopyranose and a very slow change of temperature excluding the non-controlled nucleation caused by impurities. The morphologically homogeneous crystallization depends, according to SMELÍK (1961), on the preparation of the solution suitable for crystallization, on the stabilization of the anomer replacement of D-glucose molecules, on the hydration structure, on supersaturation and on temperature.

Neither the optimum temperature prior to crystallization, nor the directed anomerization of the D-glucose molecules bring about the desired effect if

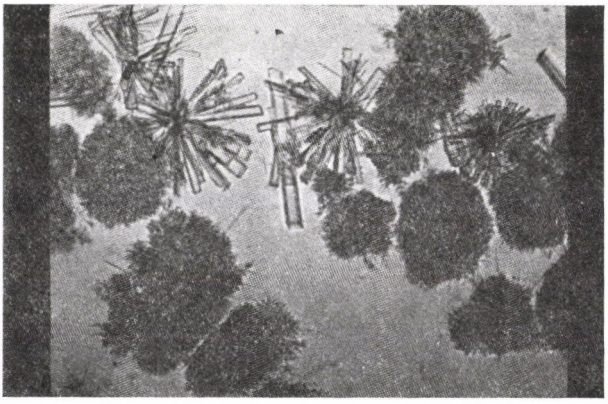


Fig. 8. Hedgehog-like needle crystals and rose resembling crystal formations crystallized in allotropically labile solutions

crystallization occurs at 20 ± 2 °C in the region of 65 ± 5% concentration and labile from the point of view of configuration. A brittle mass of non-transparent needle crystals grows around a single seed constituting a hedgehog-like formation. Around this a configuration grows slowly, consisting of lamellae limited by transparent planes, resembling a rose (Fig. 8). The lamellae-like configurations as observed by DEAN (1974), which are characteristically interlaced in this phase of crystallization, are monocline, orthorombic formations. Analyzed by gas chromatography they were found to consist of α-D-glucopyranose monohydrate and β-D-glucopyranose monohydrate.

3.7. Crystallization of α-D-glucopyranose monohydrate

SIPYAGIN (1950) distinguishes between technologically favourable and unfavourable α-D-glucopyranose crystals. The industry finds desirable the lamellated hemimorph monocline crystals of α-D-glucopyranose monohydrate which are easy to separate from the mother liquor (Fig. 9). It is very difficult even impossible to wash out the hedgehog-like formations or the very small needle crystals.

The maximum dimensions of α-D-glucopyranose monohydrate crystals may reach 1–2 mm. In case of larger dimensions cracks were observed. Favourable crystals may be obtained in the following ways:

— Spontaneous microcrystallization in a liquid film of 70–90% concentration (MEISEL, 1963; TITTELBOOM, 1968).

— Crystallization in a suspension containing 15–50% solid seed-crystals in a solution suitable to crystallization. The process can be automated (HIGASHI & KANNO, 1969; ARKHIPOVICH & PETRUSHEVSKIĬ, 1969; IDASZAK, 1960).

— Crystallization in supersaturated aqueous solutions. According to SADOVYĬ (1933) there is a linear correlation between supersaturation and rate of crystallization or the correlation is exponential in viscosity in both pure and

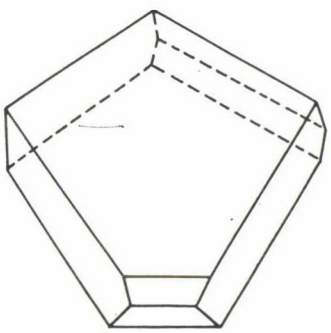


Fig. 9. Idealized drawing of laminated hemimorph monocline α-D-glucopyranose monohydrate (SIPYAGIN, 1950)

contaminated solutions. In a crystallization process during 30 h at 25 ± 5 °C temperature DEAN and PYLE (1971) obtained 50–60% yield of crystals of 0.6 mm dimensions. The crystallizing suspension was gradually added at a higher temperature to the supersaturated solution of 65% concentration. SMELÍK (1961) takes into account the dependence of crystallization rate of α-D-glucopyranose monohydrate on the cooling rate to 20 ± 5 °C. He suggests to admix the crystal suspension of 7.5 ± 2.5% seeding mass at a rate preventing sedimentation. The formation of morphologically and allotropically homogeneous crystals of 1.0 mm size is aided by substituting 5% of the water by ethyl alcohol. Thereby secondary nucleation is excluded and the development of crystals accelerated. Well developed transparent needle crystals can be obtained under laboratory conditions according to SMELÍK (1975) by the following method: a 59 ± 1% D-glucose solution is prepared at 71 ± 1 °C temperature. In order to stabilize the hydration construction and to promote more or less the anomer orientation of the molecules he adds to the freshly prepared solution (refractive index $[n]_D^{20°}$ = = 1.4360) 96% ethyl alcohol thereby replacing gradually 5% of the water present. Ethyl alcohol may be added during the cooling of the solution or when after reaching the temperature of 20 ± 5 °C the metastable supersaturation state sets in. The formation of seed crystals and the development of crystals in a well sealed vessel under occasional shaking, may take a whole week. After separation of the mother liquor the crystal mass is washed with ethyl alcohol, then dried in an air flow of 50 ± 5 °C temperature. The crystallization process as described is in accord with criteria of the new modified D-glucose – water phase diagram (Fig. 3).

Literature

ARKHIPOVICH, N. A. & PETRUSHEVSKIĬ, V. V. (1969): O nepreryvnoi kristallizatsii gidratyi glyukozy. *Sakh. Prom.*, *43*, 68–72.

CHARLES, D. F. (1959): Refractive indexes of pure sucrose solutions. *Int. Sugar J.*, *61*, 236–240.

CHARLES, D. F., (1960): Solubility of pure sucrose in water. *Int. Sugar J.*, *62*, 126–131.

DEAN, G. R. (1973): Optical – crystallographic properties of β-D-glucose. *Anal. Chem.*, *45*, 2440–2441.

DEAN, G. R. (1974): An unstable crystalline phase in the D-glucose – water system. *Carb. Research*, *34*, 315–322.

DEAN, G. R. & PYLE, R. E. (1971): *Gewinnung verhältnismässig grosser Dextrosekristallen.* German Pat., No. 2 044 681.

EBERT, CH., NEWKIRK, W. B. & MOSKOVITZ, M. (1927a): *Production of dextrose from starch.* USA Pat., No. 1 668 308.

EBERT, CH., NEWKIRK, W. B. & MOSKOVITZ, M. (1927b): *Production of dextrose.* USA Pat., No, 1 673 187.

GILLIS, J. (1923): Über die Lage des Umwandlungspunktes der Glukose. *Rec. trav. chim. des Pays-Bas*, *42*, 1077–1079.

GRUT, E. V. (1937–38): Šetřeni o přesycení a viskozitě v refrižerantech pro prvni výrobek. II. Rozpusnost sacharozy v čiste vodě. (Investigations of supersaturation and viscosity in crystallisers for white massecuites. — Part II. Solubility of sucrose in pure water.) *Listy cukrovarnické*, *53*, 53–55.

HIGASHI, S. & KANNO, T. (1969): *Crystallization of glucose.* Japan Pat., No. 6 908 838.

HOLESZOVÁ, I. (1967): *Dehydratačni efekt bezvodnej kryštalickej α-D-glukózy.* (Dehydrating effect of anhydrous crystalline α-D-glucose.) Diploma thesis, Slovak Polytechnic College, Bratislava.

HRUBÝ, R. (1937–38): Kontrola svařeni cukornych roztokú elektrickou vodivostí. I. Rozpusnost sacharózy. II. Připrava nasýcených roztoku. (Sugar boiling controll with electrical conductivity. – Part I. Solubility of sucrose. Part II. Preparation of saturated solutions.) *Listy cukrovarnické, 53,* 345–351; 404–406.

IDASZAK, L. R. (1960): *Crystallization of dextrose.* USA Patent, No. 3 440 093.

JACKSON, R. F. & SILSBEE, C. G. (1922): The solubility of dextrose in water. *Natl. Bur. Stand. Sci. Paper, 17,* No. 437, 715.

MEISEL, M. (1963): *Crystallization of α-dextrose hydrate.* French Patent, No. 1. 331, 252.

NEWKIRK, W. B. (1923): *Production of dextrose from starch.* USA Patent, No. 1 521 829.

NEWKIRK, W. B. (1924a): *Production of dextrose.* USA Patent, No. 1 521 830.

NEWKIRK, W. B. (1924b): Preparation and use of refined dextrose. *Ind. Eng. Chem., 16,* 1173–1175.

NEWKIRK, W. B. (1924c): *Dextrose hydrate.* USA Patent, No. 1 559 176.

NEWKIRK, W. B. (1924d): *Coarse granulated dextrose from starch.* ISA Patent, No. 1 640 717.

NEWKIRK, W. B. (1924e): *Production of pure dextrose from starch.* USA Patent, No. 1 658 998.

NEWKIRK, W. B. (1924f): *Preparation of pure crystalline dextrose from starch.* USA Patent, No. 1 661 898.

NEWKIRK, W. B. (1929): *Production of crystalline dextrose from a solution.* USA Patent, No. 1 722 761.

NEWKIRK, W. B. (1930a): *Production of pure dextrose from uncleaned dextrose.* USA Patent, No. 1 750 938.

NEWKIRK, W. B. (1930b): *Production of crystalline anhydrous starch sugar.* USA Patent, No. 1 976 361.

NEWKIRK, W. B. (1936a): *Dextrose crystallization.* USA Patent, No. 2 065 724.

NEWKIRK, W. B. (1936b): Developing and preparation of anhydrous dextrose. *Ind. Engng Chem., 28,* 760–766.

NEWKIRK, W. B. (1939): *Soft-grained dextrose free from water of crystallisation.* USA Patent, No. 2 141 547.

NÝVLT, J. (1967): *Kryštalizácia z roztoku.* (Crystallization from solutions.) Štátne Nakladatelstvo Technickej Literatury, Bratislava, pp. 21–22; 43.

RYBÁROVÁ, B. (1975): *Rozpustnost D-glukózy vo vode v oblasti tvorby hydrátu D-glukózy.* (Solubility of D-glucose in the range of D-glucose-hydrate formation.) Diploma thesis, Slovak Polytechnic College, Bratislava.

SADOVYĬ, I. E. (1953): Zavisimost' skorosti kristallizatsii glyukozy ot osnovnykh fizicheskikh faktorov. *Shurn. Prikl. Khim., 26,* 949–959.

SIPYAGIN, A. S. (1950): *Tekhnologiya krakhmalo-patochnogo proizvodstva* Moskva, Pishchepromizdat, pp. 371–383.

SMELÍK, A. (1961): *Kryštalizácia glycidov.* (Crystallization of sugars.) Research report, Slovak Polytechnic College, Bratislava.

SMELÍK, A. (1971): *Rozpusnosť D-glukózy vo vode v oblasti tvorby α a β modifikácie.* (Solubility of D-glucose in water in the range of the formation of α and β modifications.) Research report, Slovak Polytechnic College, Bratislava.

SMELÍK, A. (1975): *Spôsob kryštalizácie racematu D-glukózy z presýteneho vodného roztoku alebo z vodného roztoku v prítpmnosti kyseliny octovej.* (Process for the crystallization of racemic D-glucose from supersaturated aqueous solution or from aqueous solution in presence of acetic acid.) CSSR Patent, No. 156 873.

SMELÍK, A. (1980): *Spôsob kryštalizácie α-anomeru bezvodnej D-glukózy.* (Process for the crystallization of anhydrous α-D-glucose anomer.) ČSSR Patent, No. 206 080.

SMELÍK, A., IVANČENKO, D. & MATEJOVÁ, J. (1968): Index lomu vodných roztokov D-glukózy. (Refractive index of aqueous D-glucose solutions.) *Listy Cukrovarnické, 84,* 223–229.

TITTELBOOM, M. L. E. (1969): *Dextrose (D-glucose) crystallization.* USA Patent, No. 3 406 046.

YOUNG, F. E. (1957): D-Glucose – water phase diagram. *J. phys. Chem., 61,* 616–619.

Acta Alimentaria, Vol. 19 (2), pp. 157—163 (1990)

LACTIC ACID FERMENTATION OF WHOLE WHITE CABBAGE[a]

H. BUCKENHÜSKES[b, d]; H. OMRAN[c] and K. GIERSCHNER[b]

[b] Institute of Food Technology, Hohenheim University.
Federal Republic of Germany
[c] Suez Canal University, Ismailia. Egypt

(Received: 15 May, 1989; accepted: 6 September 1989)

The lactic acid fermentation of whole heads of cabbage is originated in the South-East-European countries. Since a few years the process is used in some West-European countries, too. Normally the fermentation works spontaneously in a sodium chloride containing brine at ambient temperatures. In this work the time course of the fermentation was investigated. It was found, that the changes caused by the fermentation processes depend greatly on the different parts of the cabbage tissue. In the outer leaves the pH decreased much faster than in the inner ones but at the same time, the core region showed a higher total acid content than the outer part of the cabbage. This effect may be caused by leaching of buffering substances out of the outer leaves into the brine. The vitamin C content decreased in all parts of the cabbage but in the outermost leaves to a significant extent.

Keywords: lactic acid fermentation; whole cabbage

The lactic acid fermentation of whole heads of white cabbage comes from the South–East-European countries like Hungary, Turkey or Yugoslavia. For the last few years the process has been also applied in some West-European countries, e.g. France and F. R. G. (BUCKENHÜSKES et al., 1989). The fermented product is used in several ways to prepare traditional meals, e.g. the Yugoslavian "Sarma", which is usually made by baking ground meat rolled in the fermented leaves. Industrial fermentation takes place spontaneously in a brine containing 5–6% of sodium chloride at ambient temperatures. Discussions with several producers showed, that there are two important problems in the production. The first problem is the formation of slimy substances (dextrans) which can be destroyed by that treatment. The second problem is the disintegration of the tissue near to the stalk. The only available paper related to the fermentation of whole heads of white cabbage was published by PEDERSON and co-workers (1962) and this treats the subject from the aspect of microbiology. The aim of our work was to follow the fermentation process within the cabbage.

[a] Presented at the International Conference Biotechnology and Food, Stuttgart—Hohenheim, FRG, 20–24 February 1989

[d] Present address: Gewürzmüller Dr. E. Rendler, Klagenfurter Str. 1—3, D-7000, Stuttgart 30. FRG

1. Materials and methods

White cabbage of unknown variety was obtained from a local farm. The trimmed heads were sized and placed (57.2 kg) into a fermenting tub. Eighty kg of brine (5% NaCl) was poured in and the cabbage was wedged by a stone to force the heads well below the brine surface. The tub was covered and placed in a room of 15–22 °C temperature for fermentation (34 days). During fermentation at certain intervals samples (always one head) were taken. Beginning from the outside always 6 leaves of the cabbage were removed and collected as one layer (outside = 1st layer). The homogenized and filtrated samples were analysed for pH, total acid content (titration with 0.1 *N* NaOH to pH 7.0) and sugar content (methods described by BUCKENHÜSKES, 1984). The vitamin C content was measured by a modified method according to Tillmans (BOHRER, 1986). The buffering capacity of the raw material was measured by the method of BUCKENHÜSKES and GIERSCHNER (1985).

2. Results

2.1. pH and acid content of the brine

Figure 1 shows the relationship between the development of pH and acid content in the brine during fermentation. Due to the low buffering capacity of the fresh brine the small amount of developed acid causes an obvious decrease in pH. From the 5th day of fermentation the acid content increased rapidly

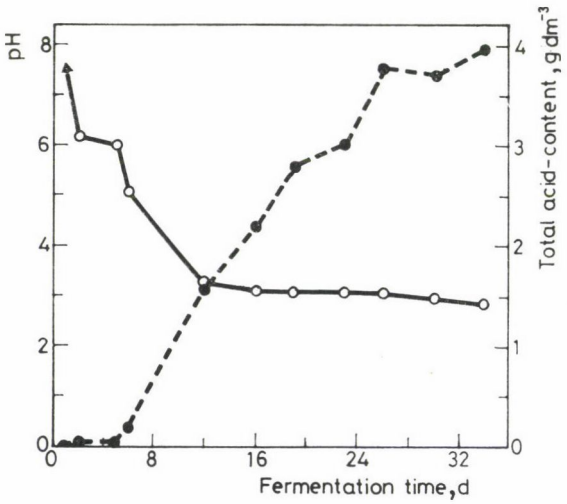


Fig. 1. pH value and total acid content of the brine during fermentation. ●-----●: pH value; ○——○: total acid

but the pH reached only a value of about 3.0. This phenomenon can be ascribed to the enrichment of the brine with soluble components from the cabbage intensifying the buffering capacity of the brine. The enrichment is caused by osmotic and diffusion processes.

2.2. pH and acid content of the cabbage leaves

Figure 2 demonstrates that the pH of the cabbage decreased very rapidly within the first days of fermentation. Upon reaching a value of approximately 3.7 further pH-decrease slowed down while the total acid content of the tissues still increased (see Table 1). This is due to the natural buffering capacity of the cabbage (BUCKENHÜSKES & GIERSCHNER, 1985). Furthermore Fig. 2 shows that the pH of the inner parts of the cabbage is higher than the pH of the outer ones. In contrast to this the total acid content increased from the surface to the core of the cabbage as it is shown in Table 1.

2.3. Sugar content of the cabbage leaves

Figure 3 shows the residual sugar content in the different parts of the cabbage in dependence of the fermentation time. It is demonstrated, that the inner leaves contain about 15 g of sugar by the end of the fermentation period whereas the sugar content of the outermost leaves has leached and was metabolized nearly quantitatively.

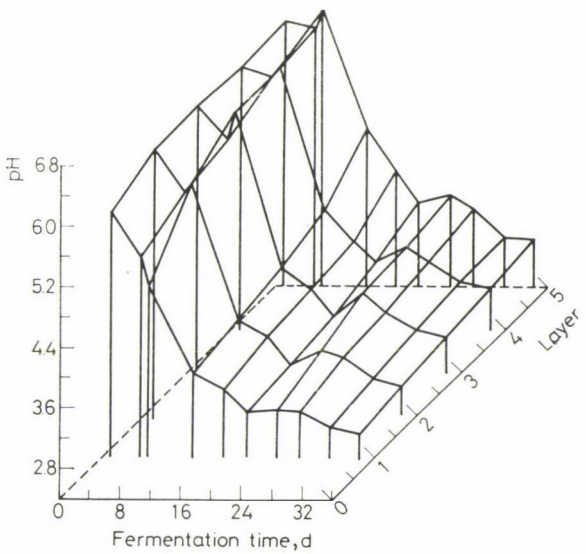


Fig. 2. pH values of the different parts of the cabbage in dependence of the fermentation time

Table 1

Total acid-content of the different parts of the cabbage in dependence of the fermentation time

Time (days)	Layer				
	1	2	3	4	5
1	0.38	0.27	0.30	0.38	0.51
3	0.38	0.27	0.27	0.27	0.27
5	0.68	0.49	0.35	0.32	0.30
6	1.11	0.49	0.32	0.30	0.32
12	4.19	4.21	4.24	3.51	1.86
16	4.62	5.89	6.40	6.80	4.56
19	3.97	5.13	6.08	7.70	7.29
23	5.67	8.86	7.78	8.26	7.88
26	5.29	8.21	8.69	9.13	8.80
30	5.40	8.96	10.31	10.48	10.69
34	4.97	8.64	9.45	9.83	11.66

2.4. Vitamin C content of the cabbage leaves

In Fig. 4 it can be seen, that the vitamin C content decreased in all parts of the cabbage but in the outermost leaves to a tremendous extent. Contrary to PEDERSON and co-workers (1962) the data of the vitamin C content in the brine show that most of the vitamin extracted is destroyed.

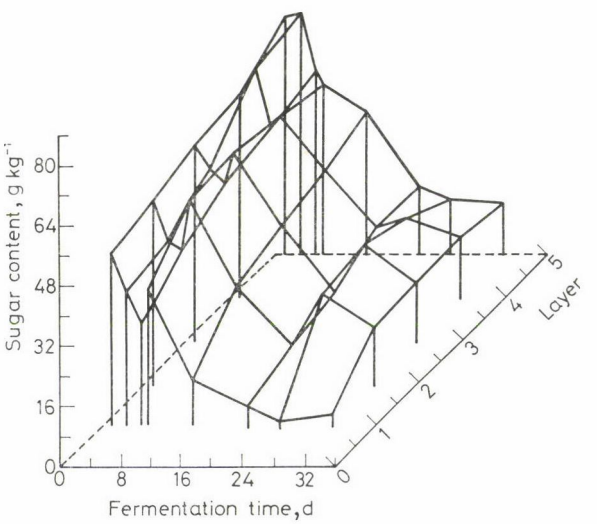


Fig. 3. Residual sugar content in the different parts of the cabbage as a function of the fermentation time

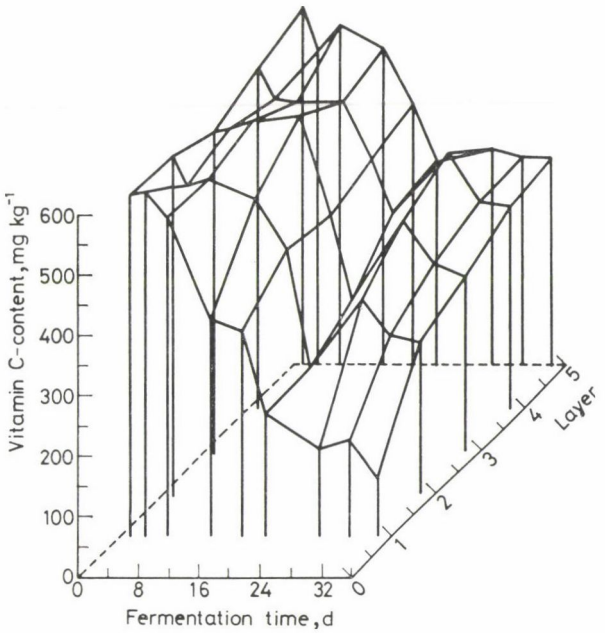


Fig. 4. Vitamin C content of the different parts of the cabbage as a function of the fermentation time

2.5. Special remarks

During fermentation the colour of the cabbage tissue changed from white to yellow and the tissue got a glassy appearance. The leaves were palatable and of mellow flavour; the texture was firm. In some cases we found a disintegration of the tissue near to the stalk as it can be seen in Fig. 5.

Fig. 5. Disintegration of the core of white cabbage

3. Conclusions

The demonstrated results of the pH and the total acid content in the different parts of the cabbage imply two phenomena which have to be discussed. The acid content of the core is in accordance with the value calculated from the natural buffering capacity of the raw material corresponding to the pH reached. In the outer leaves the final pH value corresponds to half of the calculated quantity of acid. This means that the natural buffering capacity of these leaves decreased during fermentation. This effect is brought about by extraction of buffering substances into the brine as described in connection with Fig. 1. The second phenomenon is the striking difference in the acid content of the different layers. To explain this, we think that fermentation works mostly on the surface of the cabbage leaves and in the brine which infiltrates into the space between the leaves. It seems that the metabolites of the microorganisms (lactic and acetic acid) produced in the core region mostly diffuse into the vegetable tissues. Otherwise the substances produced in the outer regions of the cabbage head are leaching more and more into the free brine resulting in an equilibrium concentration in the brine and the tissue.

The observed disintegration of the tissue in the vicinity of the stalk might be caused by undesirable microorganisms. The phenomenon can be prevented by removing a part of the cabbage cores. Sometimes the holes bored in the core are filled with sodium chloride to eliminate undesirable fermentations (personal informations of producers).

Literature

BOHRER, B. (1986): Möglichkeiten und Bedeutung der Vitamin C-Bestimmung in Weisskohl und Sauerkraut. – In: Verband der Lebensmitteltechnologen e. V.: *VLT-Seminar "Sauergemüse und Feinkost"*. Filderstadt.

BUCKENHÜSKES, H. (1984): *Untersuchungen der technologischen Rahmenbedingungen für ein neues Verfahren zur Herstellung von Sauerkraut in Kleinbehältern*. Dissertation, Hohenheim Universität, BRD.

BUCKENHÜSKES, H. & GIERSCHNER, K. (1985): Zur Bestimmung der Pufferkapazität einiger Gemüsearten. *Alimenta, 24*, (4), 83–88.

BUCKENHÜSKES, H., AABYE, H., ANDERSSON, R. & RODRIGO, M. (1989): Fermented vegetables. *Proceedings of the concluding seminar of COST 91*. Göteborg, Sweden. (In preparation.)

PEDERSON, C. S., NIKETIC, G. & ALBURY, H. M. N. (1962): Fermentation of the Yugoslavian pickled cabbage. *Appl. Microbiol., 10*, 86–89.

Acta Alimentaria, Vol. 19 (2), pp. 163—176 (1990)

FUNCTIONAL PROPERTIES OF THE FLOUR AND THE MAJOR PROTEIN FRACTION FROM SESAME SEED, SUNFLOWER SEED AND SAFFLOWER SEED

K. BOOMA and V. PRAKASH[a]

Biophysical Chemistry Unit, Food Chemistry Department, Central Food Technological Research Institute, Mysore-570 013. India

(Received: 3 October 1988; accepted: 18 January 1989)

Functional properties of the flour and the high molecular weight protein fractions from sesame seed, sunflower seed and safflower seed were investigated. The functional properties studied included nitrogen solubility, water absorption capacity, fat absorption capacity, emulsification capacity, foaming capacity and foam stability. Comparison of the nitrogen solubility curves of the three proteins shows that carmin, the high molecular weight protein from safflower seed was more soluble in water than the other high molecular weight proteins.

There is a decrease in water absorption capacity of the pure proteins as compared to the flour in all the seed systems. The fat absorption capacity of pure proteins shows only marginal changes when compared with that of the flours. The emulsifying capacity of pure proteins is nearly doubled as compared to that of the flour. However, the foaming properties of the pure protein were found to be much inferior to those of the flour. The results are interpreted keeping in view of the amino acid composition and the biophysical properties of the protein fractions.

Keywords: functional properties, total proteins, sesame proteins, sunflower proteins, safflower protein, major protein fraction

The need for an understanding of the functional properties of individual proteins in the native state or modified either physically or chemically is essential for optimising their use in specific food systems (SHUKLA, 1982; KINSELLA, 1982). Hence studies are undertaken on the functional properties of proteins from various sources such as meat, milk, oilseeds, leaves, etc. (MODLER, 1985; KHALIL et al., 1985a, b; WANG and KINSELLA, 1976). Oilseeds are probably a good source of proteins which can be extensively used in bringing about the desired functional properties in several foodstuffs. The role played by each component in the seed such as different protein fractions, residual fat, carbohydrate fraction have a pivotal role to play in determining the functional property of each flour obtained from the respective seed (BOOMA, 1988). However, very little study is available on the role of these components on the total functional properties of the flour (BOOMA, 1988).

Hence in order to understand the role played by individual components,

[a]To whom all the correspondence should be addressed:
Dr. V. Prakash
Biophysical Chemisty Unit
C.F.T.R.I., Mysore—570013. India

a study was initiated to understand the functional properties of defatted flour and isolated major protein fraction from selected oilseeds such as sesame seed, sunflower seed and safflower seed. An attempt is made to correlate the functional properties of pure proteins with the physico-chemical properties and the amino acid profile of the protein to understand their contribution.

1. Materials and methods

1.1. Materials

Authentic varieties of sesame seed, sunflower seed and safflower seed were obtained from National Seeds Corporation, Bangalore, India. The chemicals and reagents used were as follows: ammonium sulphate, sodium chloride, potassium sulphate, cupric oxide, selenium dioxide (Sarabhai Chemical, India); sodium hydroxide (Astra-IDL, India); hydrochloric acid (Ranboxy Laboratories, India); casein, sulphuric acid (E. Merck Co., India); Postman double refined groundnut oil (Ahmed Mills, India); sodium azide (Sigma Chemicals, USA); dialysis tubing 1'' diameter was obtained from M/s. Arthur Thomas Co., USA. Distilled water was used for all the experiments including dialysis to remove salts. Unless otherwise stated all the chemicals were of reagent grade only.

1.2. Methods

1.2.1. Preparation of flours

1.2.1.1. Sesame seed — Authentic varieties of sesame seeds (*Sesamum indicum* L.) sunflower seeds (*Helianthus annuus* L.) and safflower seed (*Carthamus tinctorius* L.) were procured cleaned and dried. The dried seeds were flaked (0.5 mm) in flaking machine (Aktiebolaget Kvarnmaskiner, Malmö type) and defatted with n-hexane till the fat content was less than one percent after at least six washes. The defatted material was dried in a cabinet drier at 50 °C for eight hours to remove the traces of hexane. The dried and defatted material was powdered to 60 mesh size in a Quadramat mill and used for routine extraction of proteins and other studies.

1.2.2. Isolation of high molecular weight proteins

1.2.2.1. Alpha globulin — The protein was isolated by the procedure of PRAKASH and NANDI (1978) and its homogeneity tested.

1.2.2.2. Helianthin — The protein was isolated by following the procedure of RAHMA and NARASINGA RAO (1979) and its homogeneity tested.

1.2.2.3. Carmin — The protein was isolated by following the procedure of LATHA and PRAKASH (1986) and its homogeneity tested.

1.2.3. Analytical ultracentrifugation. The homogeneity of the high molecular weight protein isolated was checked in 1 mol l^{-1} NaCl in a Spinco Model E

analytical ultracentrifuge. A standard 12 mm Kel F single sector centerpiece was used. The experiments were carried out at 27 °C and at 56 100 rp.m. using. 1% protein solution. The homogeneity was ascertained by standard procedure (SCHACHMAN, 1959).

1.2.4. Nitrogen solubility

1.2.4.1. Flour — To 1 gram of the flour weighted an Mettler PE 1600 balance 100 cm^3 of distilled water was added and the pH of the suspension was adjusted by adding 1 *N* HCl or 1 *N* NaOH using a Systronics-331 pH meter. The suspension was then shaken for one hour in a Queue orbital shaker at 150 r.p.m. and 30 °C. The slurry was centrifuged in a Sorvall-RC 5B centrifuge at 6000×g for 30 min and the pH of the supernatant was noted. Aliquots of 5 cm^3 were taken for nitrogen determination by Kjeldahl method (AOAC, 1980) and the nitrogen was estimated.

1.2.4.2. High molecular weight protein — Two hundred mg of high molecular weight protein from various oilseeds were weighed on a Mettler PE 1600 balance and dissolved in water at pH 11.5 using 1 *N* NaOH and adjusted in a Systronics 331 pH meter. The solution was centrifuged in Sorvall-RC 5B centrifuge at 7000×g for 30 min. The supernatant was monitored for nitrogen solubility by measuring absorbance in a Shimadzu double beam UV-150-02 spectrophotometer. Aliquots of 0.5 cm^3 of the supernatant were added to vials containing 9.5 cm^3 of water in the range of pH from 2–11. They were shaken in Queue orbital shaker for one hour at 150 r.p.m. and 30 °C and then centrifuged in Sorvall-RC 5B centrifuge at 7000×g for 30 min. The pH of the supernatant was measured in a Systronics 331 pH meter and absorbance measured in Shimadzu double beam UV-150-02 spectrophotometer. The absorbance at 280 nm was converted to nitrogen values using the extinction coefficients of 10.8, 11.0 and 10.0 for alpha-globulin, helianthin and carmin, respectively (PRAKASH & NANDI, 1978; RAHMA & NARASINGA RAO, 1979; LATHA & PRAKASH, 1986).

1.2.5. Functional properties

1.2.5.1. Water absorption capacity (WAC) — The water absorption capacity was determined by the method of SOSULSKI (1962) and is expressed as the amount of water (g) retained by 100 g of the material or 100 g of protein in the residue. Corrections are given to soluble solids and soluble nitrogen and are expressed as water retained by 100 g of insoluble protein. A known quantity of the flour (2 g) and in the case of high molecular weight protein 0.5 g were put in weighted centrifuge tubes and excess of water was added (20 cm^3 for flour and 10 cm^3 for protein). For acidic pH suspension the pH was adjusted to the desired value using 1 *N* HCl. The suspension was vigorously mixed for 30 min allowing 5 min rest period between each mixing using a glass rod. After centrifuging at 7000×g for 30 min in a Sorvall RC-5B centrifuge at 27 °C the supernatant was decanted and the tubes dried in an oven at a temperature of

50 °C for 10 minutes. The water absorption is calculated from the increase in weight and the soluble nitrogen in the supernatant is corrected for.

1.2.5.2. Fat absorption capacity (FAC) — This was determined by the method of SOSULSKI and co-workers (1976) and expressed as the amount of oil in bound by 100 g of the material or 100 g protein. Fat absorption was measured by adding a known amount of flour (1 g), high molecular weight protein (0.5 g) to a known amount of oil (12 cm^3 for flour and 10 cm^3 for high molecular weight protein). The contents were stirred gently using a glass rod every 5 min for 30 min and then centrifuged at 7000 × g for 30 min in a Sorvall RC-5B centrifuge. The free oil was decanted and percentage of absorbed oil determined by difference.

1.2.5.3. Emulsification capacity (EC) — The method of BEUCHAT and co-workers (1975) was used and EC expressed as cm^3 of oil emulsified by one gram of protein. A known amount of flour (2 g and high molecular weight protein (0.5 g) and a known amount of water (23 cm^3 for flour and 12.5 cm^3 for high molecular weight protein) were added to a blending jar and blended for 30 s at 2500 r.p.m. in a Bajaj Supermix blender. Refined groundnut oil (Postman) was added from a burette to the blended sample until the emulsion breakpoint was reached. The breakpoint was defined as the point when emulsion coalescence broke to yield liquid separation and substantial loss in visual consistency as described by BEUCHAT and co-workers (1975).

1.2.5.4. Foam capacity (FC) and foam stability (FS) — A known amount of the materials (4 g of sesame seed flour and 0.5 g of the high molecular weight protein) were taken after standardization for studies on foaming. The material weighed on a Mettler PE 1600 balance was transferred with 100 cm^3 of distilled water for flour and 50 cm^3 for high molecular weight protein in an electric Bajaj Supermix blender. The suspension was blended at 2500 r.p.m. for 6 min in a Bajaj Supermix. The contents were transferred to a 250 cm^3 measuring cylinder for flour and 100 cm^3 measuring cylinder for high molecular weight protein and the volume was recorded after 30 s. Foaming capacity was expressed as percent increase in volume (LAWHON et al., 1972).

$$\text{Foaming capacity} = \frac{\text{Volume after whipping-volume before whipping}}{\text{volume before whipping}} \quad (1)$$

The foam volume was recorded for two hours after whipping at regular intervals of 1 min (for first 5 min), 5 min (till 30 min), 15 min (till 1 h) and 30 min (for the rest of the period) (AHMED & SCHMIDT, 1979). Foam stability was calculated as

$$\text{Foam stability} = \frac{\text{Foam volume after 30 min}}{\text{Initial foam volume}} 100 \ldots \quad (2)$$

All measurements were made in duplicate and average of the two values reported.

1.2.5.5. Hydrophobicity — The average hydrophobicity was calculated according to the procedure of BIGELOW (1967) by calculating the total hydrophobicity from the number and nature of various amino acid residues and their energy requirement for transferring them from organic phase to aqueous phase (PRAKASH & NARASINGA RAO, 1986).

2. Results

The different flours that were prepared as explained under Materials and methods were stored at room temperature (25 °C) for a maximum period of 3 weeks under identical conditions till the functional properties were determined. The high molecular weight protein fractions isolated in various experiments as described earlier were stored in a desiccator at room temperature. The protein and moisture content of sesame seed flour, sunflower seed flour and safflower seed flour were 50% and 4.5%, 47.5% and 3.9%, 45% and 3.7% respectively.

In Fig. 1 is shown the analytical ultracentrifuge pattern of high molecular weight protein fractions viz. alpha-globulin, helianthin and carmin from sesame seed, sunflower seed and safflower seed, respectively. From the pattern it is apparent that the three high molecular protein fractions from the three different oilseeds are homogeneous with nearly 99% protein in them.

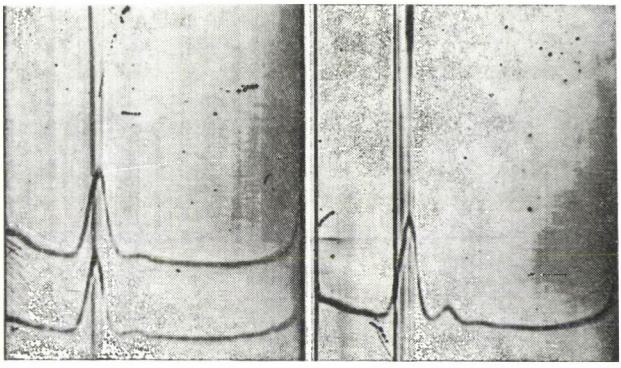


Fig. 1. Sedimentation velocity pattern of the isolated high molecular weight protein fraction from the various oilseeds. The photographs were taken 24 min after attaining the maximum speed of 59 780 rpm. The runs were performed at 26 °C. a: upper — alpha--globulin from sesame seed, lower — helianthin from sunflower seed; b: carmin from safflower seed. Sedimentation proceeds from left to right

In the above indicated two materials from each of the seeds viz. defatted flour and the pure high molecular weight protein fraction, the different functional properties were determined as indicated under Materials and methods.

2.1. Nitrogen solubility

In Fig. 2 is shown the nitrogen solubility profile as a function of pH both for total protein present in the flour as well as the high molecular weight protein from the respective flours.

In the case of sesame seed total protein, the minimum is around pH 4.6 and the results correlate well with the published literature with respect to solubility of sesame proteins (PRAKASH & NARASINGA RAO 1986; PRAKASH 1986). On the other hand the solubility profile of high molecular weight protein fraction indicates high solubility in the acidic region up to pH 4 below which

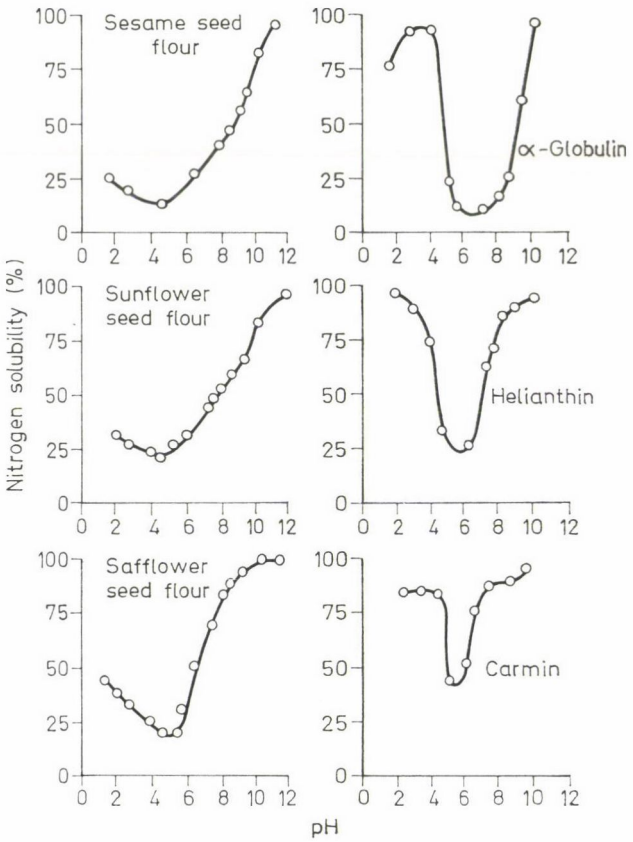


Fig. 2. Nitrogen solubility versus pH curves for sesame seed, sunflower seed and safflower seed flours and also the isolated high molecular weight protein fractions viz. alpha-globulin, helianthin and carmin, respectively, from the three respective seeds

there is a decrease in solubility (Fig. 2). These data indicate that there is an enormous difference in the solubility profile of total protein from sesame seed as compared to the high molecular weight protein fraction.

In the case of sunflower seed, the nitrogen solubility profile of the total proteins from the flour also shows a similar trend as that of sesame seed flour with minimum solubility at pH 4.5. On comparing helianthin with the total proteins of sunflower (Fig. 2) one can see the difference in the solubility profile of the total protein and of the high molecular weight protein fraction. In the alkaline region, the solubility follows similar trend but in the acidic region, the solubility of total protein is much lower compared to that of high molecular weight protein which appears to be highly soluble.

In the case of safflower seed total protein the profile even though similar to that of sesame and sunflower seed proteins with minimum solubility around pH 5, the protein is more soluble at acidic pH. Nearly 45% of the protein is soluble in the pH range of 2 as compared to 30% in case of sesame and sunflower seed proteins. Upon comparison of carmin with the high molecular weight protein fraction of other oil seeds as shown in Fig. 2 similar to other pure proteins, the solubility of carmin is quite different from that of total proteins. Further the solubility profile is not spread out in the region of minimum solubility in contrast to other proteins investigated in this study.

The above results indicate that (i) the solubility profile of the total protein of the three flours as a function of pH is different from the isolated pure high molecular weight protein fractions; (ii) the pH of minimum extractability shifts more towards the alkaline region in the case of high molecular weight proteins as compared to their respective total proteins; (iii) the pure proteins have a typical "u" shape curve and much higher solubility profile in the acidic region as compared to their respective total proteins; (iv) at extreme alkaline pH of 12 whether it is total protein or high molecular weight protein fraction all of them show very high solubility close to 90–95% of nitrogen being soluble (Fig. 2). These results indicate proper selection of the right pH for measurement of functional property and is very useful for any meaningful conclusion. Also, the pure high molecular weight protein fractions are more advantageous in the acidic region where they are more soluble compared to their respective total protein.

2.2.1. Sesame seed. In Table 1 the values for water absorption capacity of the total proteins in the flour and alpha-globulin as a high molecular weight protein are listed. The value for the high molecular weight protein is much lower than that of the total protein both at bio pH and acidic pH. However at isoelectric pH (4.5) the value for the total protein is nearly double of the value for the high molecular weight protein.

On the other hand the fat absorption capacity was similar in the flour and in alpha-globulin (Table 1). However, under similar conditions casein is

Table 1

Functional propertiess of flour, high molecular weight protein fraction alpha-globulin from sesame seed and casein at pH 6.0

Functional property	Flour	Alpha-globulin	Casein
Water absorption capacity (g per 100 g insoluble protein)			
at pH 6.0	452 ± 5	131 ± 2	160 ± 2
at pH 4.5	500 ± 5	246 ± 5	—
Fat absorption capacity (g per 100 g)	268 ± 5	248 ± 5	70 ± 2
Emulsification capacity ($cm^3\ g^{-1}$)	25 ± 2	45 ± 2	100 ± 2
Foaming capacity (% increase in volume)	81 ± 1	20 ± 2	Sparingly soluble
Foam stability (for 30 min, %)	36 ± 2	15 ± 2	Sparingly soluble
Total hydrophobicity (calculated per residues)	—	872	—

Average of 3 measurements

having a value of 70 g per 100 g protein. The emulsification capacity was nearly double for alpha-globulin as compared to 25 cm^3 for the flour (Table 1). Both values were, however, much lower than the emulsification capacity of casein (Table 1) under similar conditions. The protein alpha-globulin appears to be very poor in foaming capacity and foam stability as compared to the values for total protein which in itself has a low value in comparison with other standard proteins (KINSELLA, 1976).

The percent foam stability versus time in min is shown in Fig. 3. The total protein in the flour attains a value of nearly 36% as compared to zero time, whereas alpha-globulin attains a value of 15%. It can be seen from the Figure that the falling rate of foam stability is much higher in the case of alpha-globulin as compared to flour where the falling rate appears to be spread over a period of 60 min, whereas with alpha-globulin the process is completed in 25 min. The slow kinetics of foam destabilization may be due to two components: the high molecular weight protein where the foam collapses very fast and the 25 protein fraction (nearly 25%) which may be partly contributing to it.

These data on sesame seed proteins indicate that in most of the functional properties except emulsification capacity, the total protein in the flour gave higher value than α-globulin. The emulsification capacity of alpha-globulin had a value of 45 cm^3g^{-1} which is nearly twice as much as that of the total protein. These data indicate that the other components present in the flour such as the 25 protein fraction, low molecular weight protein fraction peptides, free amino acids, the carbohydrate fraction and to a certain extent the crude fibre may be playing an important role in determining the final value of the above mentioned functional properties.

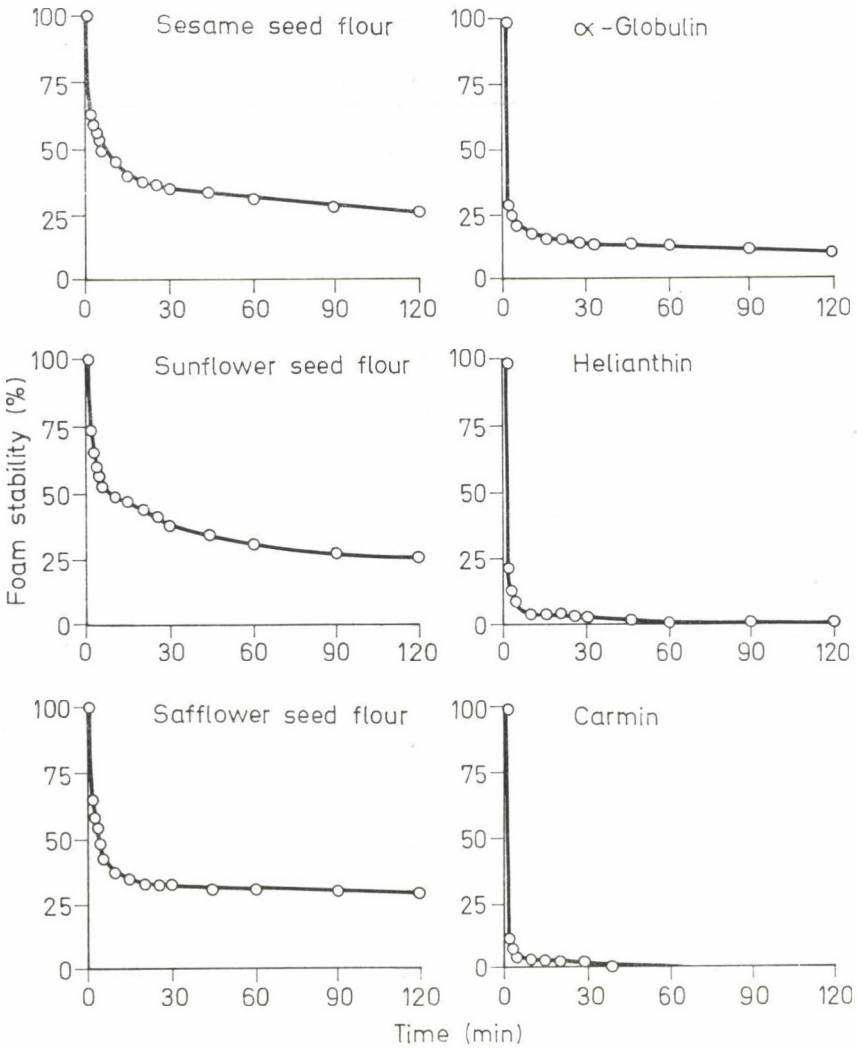


Fig. 3. Foam stability versus time curves for sesame seed, sunflower seed and safflower seed flours and also the isolated high molecular weight protein fractions viz. alpha--globulin, helianthin and carmin, respectively, from the three respective seeds

2.2.2. Sunflower seed. In Table 2 are shown the functional properties of the total protein from sunflower seed flour and the high molecular weight protein helianthin. The value of water absorption capacity for the high molecular weight protein was nearly 10 times lower than that of the total protein at bio pH. The water absorption capacity of the total protein in the flour at bio pH was nearly the same as that obtained for sesame seed total protein (Table 1). However, at acidic pH (4) a slight increase as compared to bio pH was observed with sunflower seed total protein. For the high molecular weight protein,

Table 2

Functional properties[a] *of flour, high molecular weight protein fraction helianthin from the flour of sunflower seed and high molecular weight protein fraction carmin from safflower seed at pH 6.0*

Functional property	Sunflower seed		Safflower seed	
	flour	helianthin	flour	carmin
Water absorption capacity (g per 100 g insoluble protein)				
at pH 6.0	438 ± 4	48 ± 4	600 ± 5	94 ± 2
at pH 4.0	627 ± 5	155 ± 5	645 ± 5	147 ± 7
Fat absorption capacity (g per 100 g)	229 ± 5	221 ± 5	236 ± 1	313 ± 3
Emulsification capacity ($cm^3\ g^{-1}$)	24 ± 2	44 ± 2	35 ± 2	78 ± 2
Foaming capacity (% increase in volume)	86 ± 4	20 ± 2	85 ± 4	8 ± 1
Foam stability (for 30 min, 3%)	36 ± 2	2.5	30 ± 2	1 ± 0.5
Total hydrophobicity (calculated per residues)	—	832	—	824

[a] Average of measurements

at the isoelectric pH the water absorption capacity was found to be double as compared to the value at bio pH. However, for casein the value was lower as compared to total proteins and was comparable with the pure protein at acidic pH. Significant differences were not measured between the fat absorption capacity of the flour and high molecular weight protein as found in the case of sesame seed (Tables 1, 2). However, the value was much higher than that for casein (Table 1). The emulsification capacity of the high molecular weight protein was nearly double of that of the flour. However, it was nearly half that of casein (Table 2). The foaming capacity of the total protein was found to be as good as that of sesame seed total protein. The foam stability was found to be very low for the high molecular weight protein fraction. The drop in foam stability for the high molecular weight protein fraction was very significant as compared to the high molecular weight protein fraction of sesame seed (Tables 1, 2). The drop in foam stability was very rapid reaching 2.5% in a period of 30 min.

Thus, the total proteins in flour were found to have better functional properties than the high molecular weight protein fractions. Also helianthin was found to have very poor foam stability but good emulsification property compared to that of total proteins in the flour as found in the case of sesame seed.

2.2.3. Safflower seed. In Table 2 are shown also the functional properties of the flour and high molecular weight protein carmin. The water absorption capacity of the total protein was found to be very high compared to that of high molecular weight protein carmin. The water absorption capacity of the total protein was found to be very high compared to that of high molecular weight protein carmin as found in the case of sesame seed and sunflower seed

(Tables 1, 2). The increase in water absorption capacity in the case of total protein and high molecular weight protein at acidic pH 5 was slightly higher than the value obtained at bio pH. The water absorption capacity of the total protein was nearly 6 times as high as that of casein (Tables 1, 2). The water absorption capacity of casein was comparable with the water absorption capacity of high molecular weight protein at acidic pH (Table 2). The fat absorption capacity of the high molecular weight protein was much higher than that of the flour which was different from the results of sesame seed and sunflower seed (Tables 1, 2). The fat absorption capacity of flour and carmin was higher than that of the casein (Table 2). The emulsification capacity of the high molecular weight protein was more than double of the value for flour.

Comparing the emulsification properties of three high molecular weight proteins, carmin was found to have the highest emulsification capacity. Unlike with alpha-globulin and helianthin, the foaming capacity was found to be very poor. The foaming capacity of carmin expressed as percentage increase in volume was found to be 8% as compared to 20% in the case of alpha-globulin and helianthin. The foam stability of the high molecular weight protein carmin was found to be poorer than in the case of alpha-globulin and helianthin. The foam drops to nearly zero within a period of thirty min. Comparing the functional properties of the total proteins in the flour and high molecular weight protein carmin, the flour was found to have better water absorption capacity and foaming properties. The emulsification property and fat absorption capacity of carmin was better than that of alpha-globulin and helianthin. Normally protein isolates have better emulsification capacity than their respective flours (KINSELLA, 1976).

3. Discussion

In functional properties parameters like solubility is related to properties of charge density, charge frequency and hydrophobicity (NAKAI, 1983) which determines the solubility profile of the protein in question. In the present investigation the solubility profile of the total protein and the high molecular weight protein fractions appears to be quite different (Fig. 2) which is also reflected in the water absorption capacity either at bio pH or at acidic pH for the flour and high molecular weight protein. For example, in the case of sesame flour, the water absorption capacity value of 452 g per 100 g of insoluble protein is much higher as compared to a value of 131 g per 100 g of insoluble protein for high molecular weight protein at bio pH. This result is very significant which might indicate a high hydrophobicity and lower charge frequency of high molecular weight protein as compared to the total protein from the flour. The higher charge frequency in the flour may be contributed by the low molecular weight proteins present in all the oilseeds investigated in this study

(PRAKASH, 1988). Data in the literature on the amino acid composition of low molecular weight protein i.e. the 2S fraction contains nearly 50% of glutamic acid including glutamine (PRAKASH, 1988). This implies that there is a need for the determination of functional properties of the low molecular weight proteins under homogeneous conditions of the protein. The contribution by other components of the flour such as the nature and content of carbohydrate cannot be overlooked especially when one is discussing water absorption capacity. Also, it is to be emphasized that the water absorption capacity will have more error incorporated in its evaluation if a protein is highly soluble in water. Perhaps other methods of estimating water absorption capacity, like using heavy water, needs to be explored.

The results in Tables 1–2 with respect to the fat absorption capacity indicate that there is no significant change in the fat absorption capacity of either the total protein or the high molecular weight protein showing the minor role played by other components in the total protein. Even though it is emphasized that the mechanism of fat absorption is mostly attributable to physical entrapment of oil and bulk density the role played by surface hydrophobicity and total hydrophobicity of the protein cannot be overlooked (NAKAI, 1983). The individual hydrophobicity values for the various high molecular weight proteins investigated are shown in Tables 1, 2. These values are close enough and at the same time indicate that the high molecular weight proteins belong to hydrophobic class of proteins and cannot be considered as highly charged molecules unlike the low molecular weight components in the total proteins (PRAKASH & NARASINGA RAO, 1986). Since the high molecular weight proteins are the major components (60–70%) in all the above mentioned proteins, the data obtained here can be explained in terms of the observation of the physico-chemical properties of the protein. It is to be noted that the values could differ depending upon the nature of the oil used for the experiment which could also play an important role. However, the value for fat absorption capacity appears to be much higher in the case of seed proteins than in casein (Table 2). This may have a high utility in the preparation of products such as sausages wherein the incorporation of oilseed flour may be much more beneficial than casein both from the functional and from the economic point of view.

Normally emulsification capacity, emulsion stability and activity are determined in investigating the emulsifying properties of proteins (KINSELLA, 1976). However, the evaluation of emulsifying capacity is the method most commonly adopted in the various studies. From Tables 1–2 one can see that the high molecular weight proteins have nearly twice the emulsification capacity than the proteins from the flour. For instance the emulsifying capacity of alpha-globulin is 45 cm^3g^{-1} while that of the total protein in the flour is 25 cm^3g^{-1}. This implies that eventhough the fat absorption capacity is the same for the total proteins and high molecular weight proteins as seen from

the earlier results the retention of fat in an emulsifiable form is much higher in the case of high molecular weight proteins as compared to the respective total proteins. This further indicates that whenever emulsification capacity is a criterion, it is more appropriate to use the fractionated protein if not in a 100% homogeneous form at least as an enriched fraction since this functional property is twice as high in the purified form. However, either of the values are lower compared to the emulsifying capacity of casein. These data may have a bearing on the application of such proteins in most emulsions. However, the experimental conditions such as equipment design, shape of the container, temperature, speed of blending, nature of blades in the blender, rate and mode of oil addition, pH, protein source, solubility, concentration, presence of salt and sugar and water would all individually contribute to the emulsifying capacity of proteins (KINSELLA, 1976).

With the advent a number of surfactants which have a very high foaming capacity and foam stability, the need for using edible proteins (polyelectrolytes) as foaming agents is questioned many times. But the restriction of using surfactants in food systems and the removal of several surfactants from the Gras list, has posed a new problem to the food industries in looking for foaming agents which do not contain toxicity. Proteins from oil-seeds fit in very rightly here to be utilized for their excellent foaming properties and have even the advantage of being nutrients at the same time (KINSELLA, 1976; BOOMA, 1988). In Tables 1–2 are shown the foaming capacity and foam stability values for the total proteins in the flour and high molecular weight proteins. From the data it can be seen that the high molecular weight proteins do not have any foaming capacity or foam stability as compared to the total protein. For example carmin has a foaming capacity of 8% as compared to 85% volume increase for the total protein. The trend in the data is the same for foam stability (Tables 1, 2). This shows that the total proteins in this presence of the flour (i.e. other components such as carbohydrate) exhibit an excellent capacity to encapsulate air by surrounding the air bubbles with layers of cohesive protein which have sufficient mechanical strength to prevent coalescence or rupture of bubbles in contrast to the purified high molecular weight protein fraction. Similarly to the emulsifying capacity, the experimental parameters especially the nature of the equipment, pH, temperature, concentration of the protein highly affect the foaming capacity of the protein in question. The stabilization of the foam thus formed depends upon the nature and content of stabilizers like soluble polysaccharides which contribute to the viscosity of the system ultimately stabilizing the foam. However, it is to be emphasized that attempts are made to chemically modify the proteins in a way to increase emulsification capacity, foam capacity and foam stability to the desired optimum level.

The above investigation was an attempt to indicate the role played by the major protein fractions in a composite flour to the desired functional

property in a food system. An understanding of the functional properties of pure protein systems with respect to their conformation, surface properties, physico-chemical properties would ultimately help in modifying the existing proteins to improve their functional property both in the traditional foods as well as its use in new food products.

Literature

AHMED, E. M. & SCHMIDT, R. H. (1979): Functional properties of peanut and soybean proteins as influenced by process method. *Peanut Sci., 6*, 1–6.

AOAC (1980): *Official methods of analysis.* Association of Official Agricultural Chemists. 13 Ed., Washington DC.

BEUCHAT, L. R., CHERRY, J. P. & QUINN, M. R. (1975): Physicochemical properties of peanut flour as affected by proteolysis. *J. agric. Fd Chem., 23*, 616–618.

BIGELOW, C. C. (1967): On the average hydrophobicity of proteins and the relation between it and protein structure. *J. theor. Biol., 16*, 187–196.

BOOMA, K. (1988): *Relationship between functional and physicochemical properties of proteins with special reference to seed proteins.* M. Sc. dissertation (Food Technology). University of Mysore, Mysore, India.

KHALIL, M., RAGAB, M. & ABD-EL-AAL, M. H. (1985a): Foaming properties of oilseed proteins. *Nahrung, 29*, 201–207.

KHALIL, M., RAGAB, M. & HASSANIEN, F. R. (1985b): Some functional properties of oilseed proteins. *Nahrung, 29*, 275–282.

KINSELLA, J. E. (1976): Functional properties of proteins in foods: A survey. *Critical Rev. Fd Sci. Nutr., 7*, 219–280.

KINSELLA, J. E. (1982): *Relationships between structure and functional properties of food proteins in food proteins.* — in: FOX, P. F. & CONDON, J. J. (Eds.) *Food proteins.* Applied Science Publishers, London, pp. 51–103.

LATHA, T. S. & PRAKASH, V. (1986): Isolation and characterisation of the high molecular weight protein fraction (carmin) from safflower seed (*Carthamus tinctorius* L.) *Nahrung, 30*, 833–844.

LAWHON, J. T., CATER, C. M. & MATTIL, K. F. (1972): A comparative study of the whipping potential of an extract from several oilseed flours. *Cereal Sci. Today, 17*, 240–244.

MODLER, H. W. (1985): Functional properties of non fat dairy ingredients — A review. Modification of products containing casein. *J. Dairy Sci., 68*, 2206–2214.

NAKAI, S. (1983): Structure–function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *J. agric. Fd Chem., 31*, 676–683.

PRAKASH, V. (1986): Effect of sodium chloride on the extractibility of proteins from sesame seed (*Sesamum indicum* L.). *J. agric. Fd Chem., 34*, 256–259.

PRAKASH, V. (1988): Is there a structural homology amongst the low molecular weight proteins from various oilseeds? Paper presented at the *3. Food Protein Symposium: Interactions in protein systems.* Reinhardsbrunn. GDR.

PRAKASH, V. & NANDI, P. K. (1978): Isolation and characterisation of alpha-globulin of sesame seed (*Sesamum indicum* L.) *J. agric. Fd Chem., 26*, 320–324.

PRAKASH, V. & NARASINGA RAO, M. S. (1986): Physico-chemical properties of oilseed proteins. *CRC Critical Rev. Biochem., 20*, 265–363.

RAHMA, E. H. & NARASINGA RAO, M. S. (1979): Characterization of sunflower proteins. *J. Fd Sci., 44*, 579–582.

SCHACHMAN, H. K. (1959): *Ultracentrifugation in biochemistry.* Academic Press, New York.

SHUKLA, T. P. (1982): Chemical modification of proteins. — in: CHERRY, J. P. (Ed.) *Food protein deterioration mechanisms and functionality.* ACS Symposium Series, 206, pp. 275–300.

SOSULSKI, F. W. (1962): The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem., 39*, 344–349.

SOSULSKI, F. W., HUMBERT, E. S., BUI, K. & JONES, J. D. (1976): Functional properties of rapeseed flours, concentrates and isolates. *J. Fd Sci., 46*, 1349–1353.

WANG, J. C. & KINSELLA, J. E. (1976): Functional properties of novel proteins: Alfalfa leaf proteins. *J. Fd Sci., 41*, 286–290.

Acta Alimentaria, Vol. 19 (2), pp. 177—185 (1989)

MICRONUTRIENT COMPOSITION IN SEVERAL PORTIONS OF CAPSICUM PLANTS AND THEIR RELATION TO RED FRUIT COLOUR

F. MARTINEZ-SANCHEZ[a], J. L. GIMENEZ[b], M. A. MARTINEZ-CANADAS[a], J. PASTOR[a] and C. F. ALCARAZ[b]

[a]Escuela Universitaria de Ingeniería Técnica Agrícola. Universidad Politécnica de Valencia. Orihuela (Alicante). Spain
[b]CSIC. Centro de Edafología y Biología Aplicada del Segura. P.O. Box 195. 30080-Murcia. Spain

During five consecutive years, both the concentration and the content of iron, manganese and zinc of whole red pepper plants and in three plant portions (stems + roots, leaves + petioles and fruits + seeds + stalks) were related to the red colour of the paprika obtained from the corresponding experimental plots. Only iron shows significant relations with fruit red colour level in concentration (stem and leaf portions), as well as in content (whole plant and stem portions). Manganese and zinc (in concentration and in content) are not related to red fruit colour. These results support the hypothesis of the important role of iron in the carotenoid synthesis in red pepper crops and consequently on the paprika quality, meanwhile the role of manganese and zinc can be more important in some previous processes, as flowering and fruit setting, but have not incidence on the level of red colour of the fruits.

Keywords: iron, manganese, zinc, paprika, quality

In the last four decades the traditional paprika varieties of Murcia's land, as Albar, Tres Cascos, Ramillete and Bola Americana (VIVANCOS, 1964) showed an important decrease in the level of red colour of their fruits. For this reason, three of these paprika varieties (Albar, Ramillete and Tres Cascos) practically have disappeared and an other one (Bola Americana) restricted upon to 25% of its area.

The selection and breeding of several new hybrid varieties (Horticultural Dept. CRIA, Murcia) with high colour level (Dacano, Amler, Datler, Bucano, Albar × Negral × Negral, etc.) actually offer the possibility of substitution of those traditional varieties with these new plants.

But the production of these new hybrid varieties has not elucidated the cause of the degradation of the fruit colour of the ancient varieties and they run the risk of future problems in the same sense.

During the last 15 years we have studied in cooperation with breeding researchers (ALCARAZ et al., 1982) several crop conditions and fertilizer supports that could prevent the above mentioned fruit degradation. The results (GIMENEZ et al., 1987; ROMOJARO et al., 1984) for paprika and other plants

(ALMELA et al., 1983; 1987; ALMELA & LOPEZ-ROCA, 1985) indicate the important role of some microelements on the synthesis of photosynthetic pigments and on several aspects of fruit quality.

In recent papers (ALCARAZ et al., 1988; GIMENEZ et al., 1988; MARTINEZ-SANCHEZ et al., 1988) we reported two interesting aspects: Varieties of high colour level have an important increase of the iron concentration in stems and leaves. The absorption rhythms of the plant for Fe, Mn and Zn show different trends during the vegetation period. The highest levels of Mn and Zn absorptions were observed during flowering and fruit setting. The absorption of Fe was low in the same period, but strongly increased during development and ripening of the fruit.

Considering these data, in this communication we present the results of the relations between several micronutrient levels and fruit red colour, as an other evidence of the important role of some micronutrients in the fruit pigment synthesis.

1. Materials and methods

1.1. Plant

The tested paprika cultivars (*Capsicum annuum*, L.) were: Bola Americana, Belrubi, Dacano and two Amler hybrids (B51 and B51E81). Data of several micronutrient contents and the fruit colour were obtained in 1985, 1986 and 1987 from capsicum plants grown under drip irrigated system as well as under normal land conditions in several zones of Murcia.

1.2. Sampling

During the September–October period, five plants of each experimental plot were sampled. In the laboratory, each plant was separated in the following portions: stems (stems + roots), leaves (leaves + petioles) and fruits (fruit + + seeds + stalks). Each constituted an elemental sample. Data in the table and figures are the averages of the five plant portions analyzed.

1.3. Analytical determinations

Iron, manganese and zinc were measured by atomic absorption spectrometry. Paprika red colour was determined by means of a modification of the method of BENEDEK (1958), from the integral fruit blend and expressed as capsanthene.

Table 1

Concentration and content of iron, manganese, zinc and paprika red colour in three parts of whole capsicum plants: Stems (with roots), leaves (with petioles) and fruits (with seeds and stalks). Paprika red colour is expressed as capsanthene

Concentration																	
Stems (ppm, d.m.)						Leaves (ppm, d.m.)						Fruits (ppm, d.m.)				Capsanthene (g per kg)	
Fe		Mn		Zn		Fe		Mn		Zn		Fe		Mn		Zn			
$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
180	11	23	3	20	2	199	20	75	8	61	5	59	4	22	2	23	3	2.76	0.14
116	10	38	5	16	2	235	22	98	10	46	4	71	5	23	2	40	3	3.28	0.17
779	55	21	3	14	1	226	21	52	4	42	4	35	2	10	1	21	2	3.95	0.20
271	19	12	1	9	1	262	23	71	6	51	6	51	4	13	1	22	2	3.80	0.20
305	23	13	1	20	3	254	22	58	6	32	3	34	3	11	1	25	2	3.43	0.16
1293	84	34	4	23	2	450	41	72	8	65	6	80	6	16	2	33	3	4.92	0.23
940	63	23	2	18	2	500	45	73	7	59	6	55	4	12	1	26	3	5.25	0.28
1094	78	37	5	22	3	573	50	82	9	59	5	68	6	12	1	25	2	5.52	0.30
493	30	58	7	28	3	229	20	91	9	59	7	53	4	18	2	31	2	3.20	0.17

Content (mg per plant)												
Stems			Leaves			Fruits			Total plant			Red colour as capsanthene
Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	
7.0	1.2	1.0	10.0	3.8	3.1	7.8	2.9	3.1	24.8	7.9	7.1	1496
5.2	1.7	0.7	11.5	10.7	2.3	6.4	2.1	3.6	23.1	14.5	6.6	1778
23.2	0.6	0.4	2.1	0.5	0.4	3.7	1.0	2.2	29.0	2.1	3.0	2354
16.9	0.8	0.6	4.4	1.9	0.9	6.1	1.6	2.7	27.4	4.3	4.2	2265
18.7	0.8	1.2	8.0	1.8	1.6	3.4	1.1	2.5	30.1	3.7	5.3	1859
38.2	1.0	0.7	12.1	1.9	1.7	5.4	1.1	2.2	55.7	4.0	4.6	2189
39.5	1.0	0.8	17.8	2.6	2.6	6.5	1.4	3.1	63.8	5.0	6.5	3492
56.8	1.9	1.1	20.6	2.9	2.1	8.9	1.6	3.3	86.3	6.4	6.5	4433
7.6	0.6	0.2	22.7	2.1	0.5	3.7	1.3	1.6	34.0	4.0	3.3	1734

$\bar{x}$: mean value of five samples
±s: standard deviation

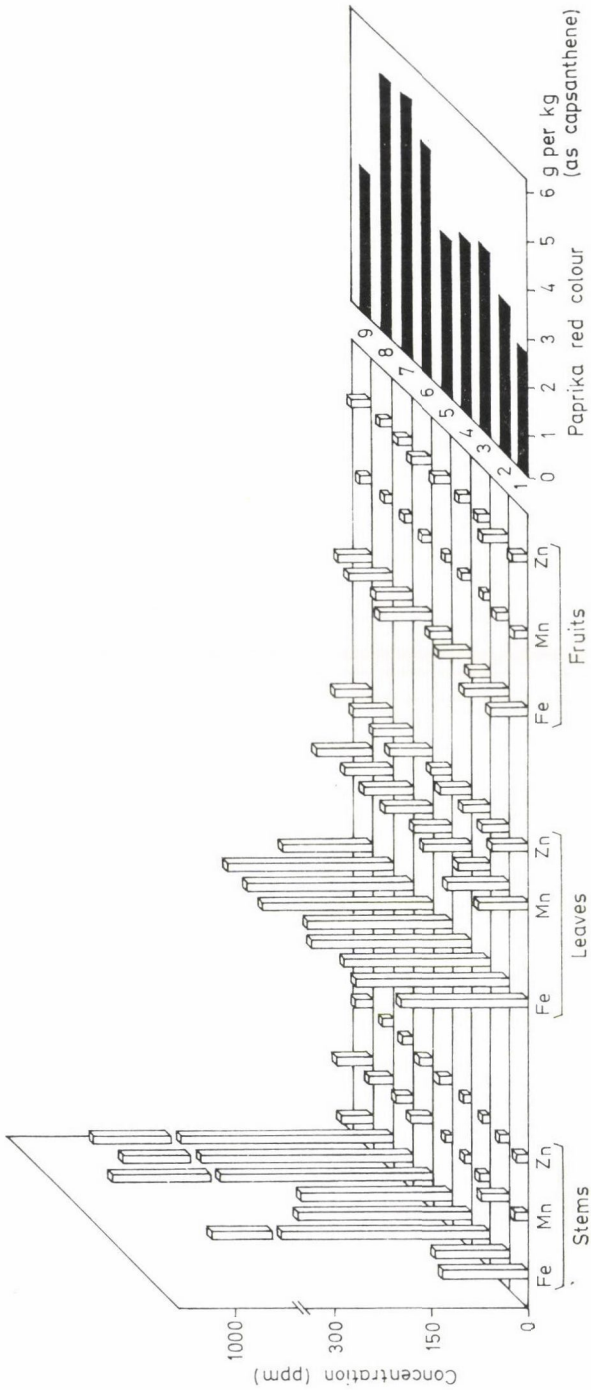


Fig. 1. Concentration of iron, manganese, zinc and paprika red colour in three parts of whole capsicum plants: stems (with roots), leaves (with petioles) and fruits (with seeds and stalks). Paprika red colour is expressed as capsanthene

2. Results and discussion

Table 1 and Figs. 1 and 2 show the results for concentration and content of Fe, Mn, Zn and red fruit colour (as capsanthene) from the different plant portions. The wide dispersion of the data is due to the different crop location, crop years, irrigation system and plant varieties. Because of this, a statistical study was carried out, concerning correlation between fruit colour and concentrations and contents of Fe, Mn and Zn in several plant parts. The results are shown in Table 2. Only some concentrations and contents of iron offer significant correlations against red fruit colour levels. These results confirm the hypothesis of GIMENEZ et al. (1988) and MARTINEZ-SANCHEZ et al. (1988) about the influence of this micronutrient on the pigment synthesis and, consequently, on the level of capsanthene of the capsicum fruits, which is the dominant red pigment of these fruits (PAVISA et al., 1987).

Manganese and zinc are important in the flowering and the fruit setting processes (GIMENEZ et al., 1988), but their plant levels at the end of the crop cycle, are not related with fruit colour. These data seem to indicate that these micronutrients have more importance in the cell multiplication processes than

Table 2

Regression coefficients and significance against zero from the linear correlations between paprika red colour and some micronutrients from several capsicum plant portions

Y	*X*	*r*	Significance
Paprika red colour concentration (g per kg)	Stem-Fe (ppm)	0.8697	**
	Leaf-Fe (ppm)	0.9549	***
	Fruit-Fe (ppm)	0.3226	N. S.
	Stem-Mn (ppm)	0.0133	N. S.
	Leaf-Mn (ppm)	0.1066	N. S.
	Fruit-Mn (ppm)	0.5576	N. S.
	Stem-Zn (ppm)	0.0475	N. S.
	Leaf-Zn (ppm)	0.3669	N. S.
	Fruit-Zn (ppm)	0.0848	N. S.
Paprika red colour content (mg per plant)	Stem-Fe (mg)	0.9033	***
	Leaf-Fe (mg)	0.7152	*
	Fruit-Fe (mg)	0.5271	N. S.
	Total plant-Fe (mg)	0.9067	***
	Stem-Mn (mg)	0.4627	N. S.
	Leaf-Mn (mg)	0.1821	N. S.
	Fruit-Mn (mg)	0.2380	N. S.
	Total plant-Mn (mg)	0.1329	N. S.
	Stem-Zn (mg)	0.2982	N. S.
	Leaf-Zn (mg)	0.1637	N. S.
	Fruit-Zn (mg)	0.3497	N. S.
	Total plant-Zn (mg)	0.2737	N. S.

* Significant at P = 95% probability level
** Significant at P = 99% probability level
*** Significant at P = 99.9% probability level
N. S.: Non-significant

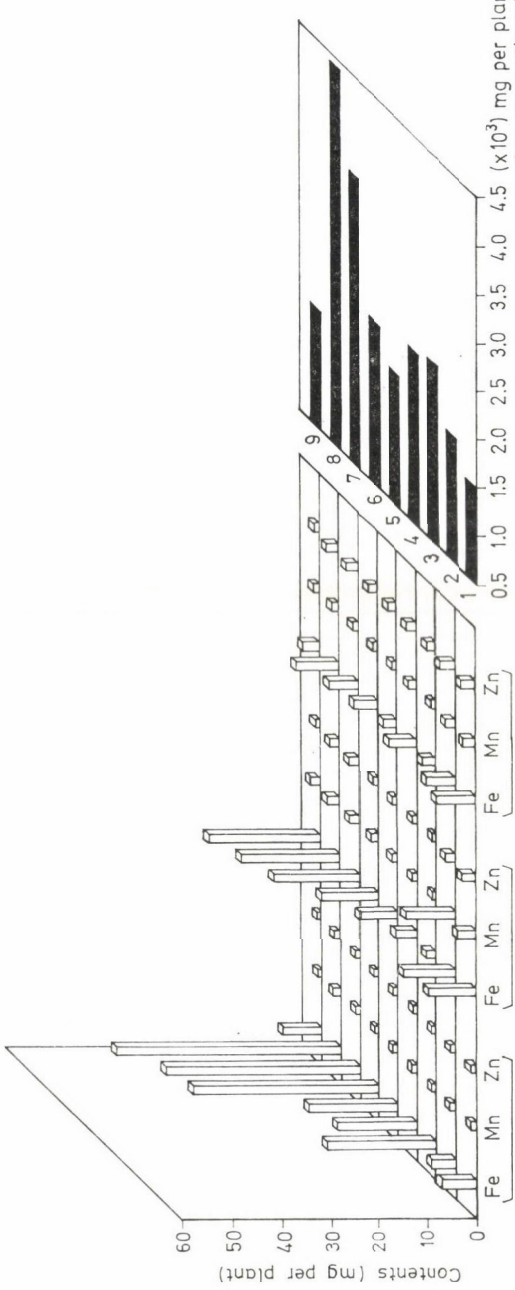


Fig. 2. Contents of iron, mangenese, zinc and paprika red colour in three parts of whole capsicum plants: stems (with roots), leaves (with petioles) and fruits (with seeds and stalks). Paprika red colour is expressed as capsanthene

in fruit quality. This hypothesis can explain their highest absorption rhythm by plants in the spring period.

Figures 3 and 4 show the correlations between iron and red fruit colour concentrations and contents, respectively. The highest significance of leaf-Fe/red colour in concentration and in total contents could indicate that plants need a minimum active-Fe concentration to enhance the red pigment synthesis. This criterium could be supported by the lack of correlation between fruit-Fe

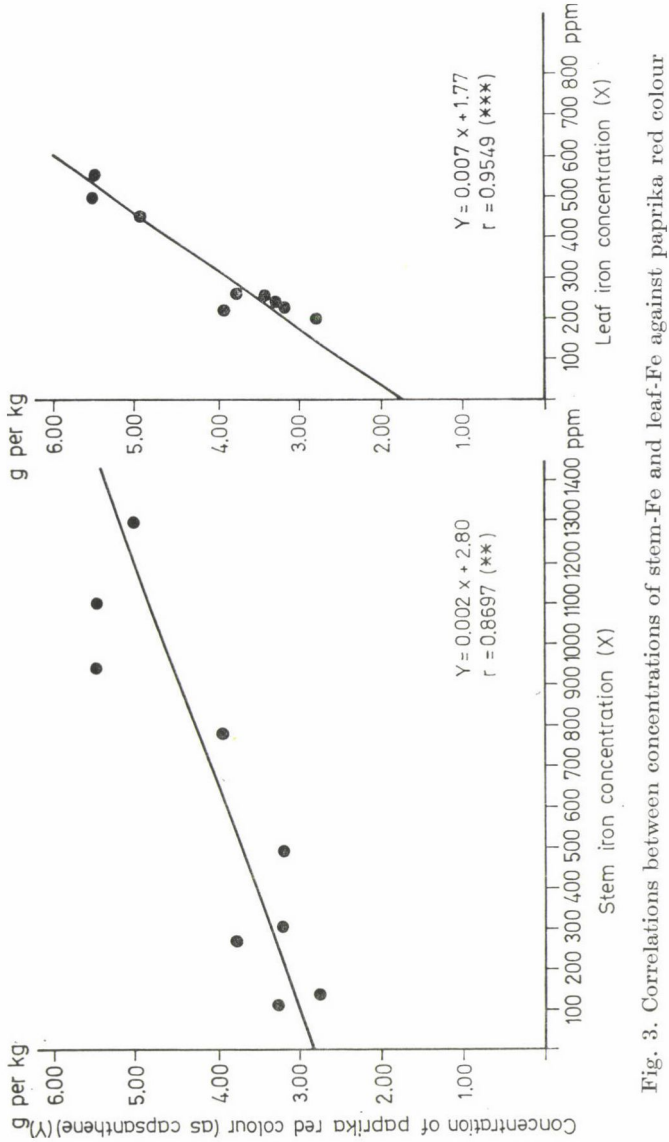


Fig. 3. Correlations between concentrations of stem-Fe and leaf-Fe against paprika red colour

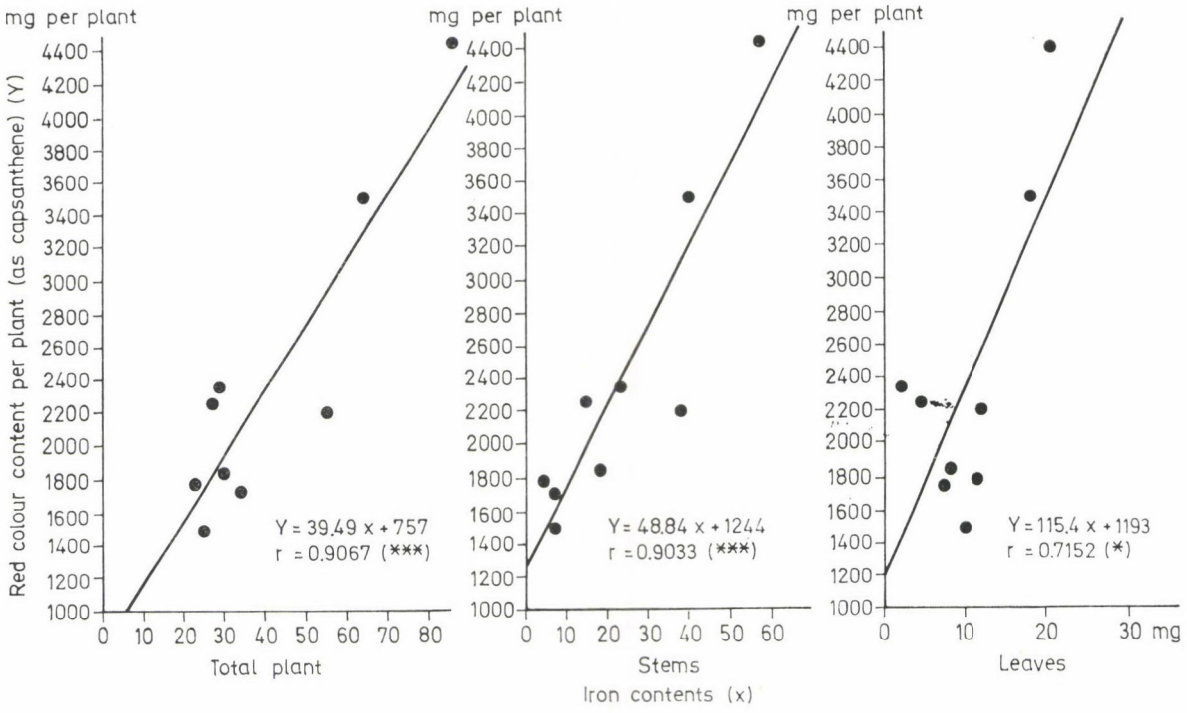


Fig. 4. Correlations between plant contents of total plant-Fe, stem-Fe and leaf-Fe against paprika red colour

and red fruit colour levels. (We think that the pigment synthesis occurs in the leaves and is then translocated to the fruits.)

Values for paprika red colour lower than 4.5 g per kg (equivalent to 150 A.S.T.A. units) are considered as characteristic of a low quality for the spanish paprika industry, and it is interesting to note that this colour level need, in our experiment, stem-Fe and leaf-Fe concentrations higher than 900 ppm and 400 ppm, respectively. These iron concentration only are reached by the Amler varieties. Considering that all the experimental plots received the same iron fertilization, these results induce us to conclude that only some Fe-efficient varieties are able to absorb enough iron for producing a suitable paprika red colour in their fruits.

Literature

ALCARAZ, C. F., ALEMAN, S., COSTA, J. C., FERNANDEZ, J., GIMENEZ, J. L., LLORENTE, S. & ROMOJARO, F. (1982): *Pimiento para pimenton*. ALCARAZ, C. F. (Ed.) Murcia. 141 pp.

ALCARAZ, C. F., MARTINEZ-SANCHEZ, F., GIMENEZ, J. L., PASTOR, J., HELLIN, E. & LLORENTE, S. (1988): Estudio de nuevas variedades de pimiento en fertigación. Part I. Absorción y distribución de macronutrientes. — in: LEAL, A., GOMEZ, M. & LLUCH, C. (Eds) *Nutrición mineral de las plantas*. Granada. pp. 79–85.

ALMELA, L., GARCIA, A. L. & MADRID, R. (1983): Deficiencia de hierro en plantas de maíz. Efecto sobre la actividad clorofilasa, clorofilas, feofitinas y proteinas en hoja. *Agrochimica, 28*, 123–131.

ALMELA, L. & LOPEZ-ROCA, J. M. (1985): Effects of iron stress on the greening of dark grown maize plants. Part. I. Quantitative aspects of the determination of chlorophyllase activity. *Photosynthetica, 19*, 37–42.

ALMELA, L., ALCARAZ, C. F., LOPEZ-ROCA, J. M., SEVILLA, F. & FERNANDEZ, J. A. (1987): Actividad clorofilasa en plantas de maíz desarrolladas en la oscuridad bajo estrés de hierro. — in: ALCARAZ, C. F. (Ed.) *Micronutrientes en Biología Vegetal y Agricultura*. Murcia, pp. 142–150.

BENEDEK, L. (1958): Untersuchungsverfahren zur Bestimmung des Farbstoffgehaltes in Paprikamahlgut. *Z. Lebensmittelunters. u. -Forsch., 107*, 228–232.

GIMENEZ, J. L. MARTINEZ-SANCHEZ, F., LLORENTE, S. & ALCARAZ, C. F. (1987): Consumo de nutrientes y su distribución en la planta en variedades de pimiento pimentonero. *Actas VII Reunión Nacional de la S.E.F.V.* Oviedo. p. 214.

GIMENEZ, J. L., MARTINEZ-SANCHEZ, F., LLORENTE, S., PASTOR, J., HELLIN, E. & ALCARAZ, C. F. (1988): Estudio de nuevas variedades de pimiento en fertigación. Part. II. Absorción y distribución de micronutrientes. — in: LEAL, A., GÓMEZ, M. & LLUCH, C. (Eds.) *Nutrición Mineral de las Plantas*. Granada. pp. 235–243.

MARTINEZ-SANCHEZ, F., GIMENEZ, J. L., HELLIN, E., LLORENTE, S., PASTOR, J. & ALCARAZ, C. F. (1988): Estudio de nuevas variedades de pimiento en fertigacion. Part III. Desarrollo vegetativo en relación con la cosecha y la calidad del fruto. — in: LEAL, A., GÓMEZ, M. & LLUCH, C. (Eds.) *Nutrición Mineral de las Plantas*. Granada. pp. 329–336.

PAVISA, A. CS., HAJDÚ, F., HOSCHKE, Á., BODNÁR, J. & BIACS, P. (1987): High-performance liquid-chromatographic control of thestandard specification of ground paprika. *Acta Alimentaria, 16*, 129–142.

ROMOJARO, F., GIMENEZ, J. L., LLORENTE, S. & ALCARAZ, C. F. (1984): Consumption of bioelements in red pepper crops. — in: MARTIN-PREVEL, P. (Ed.) *Optimization of plant nutrition*. AIONP/GERDAT. Montpellier, Vol. II, pp. 531–538.

VIVANCOS, I. (1964): Estudio de las variedades de pimientos pimentoneros propias de la cuenca del río Segura. *Agricultura, 23*, 71–76, 141–147.

Acta Alimentaria, Vol. 19 (2), pp. 187–198 (1989)

INFLUENCE OF PASTEURIZATION TIME AND TEMPERATURE ON THE RHEOLOGY AND SENSORY PROPERTIES OF A TYPE OF GAZPACHO

L. JIMENEZ and A. LOPEZ

Departamento de Ingeniería Química, Facultad de Ciencias, Universidad de Córdoba, c/San Alberto Magno s/n, 14 005 — Cordoba. Spain

(Received: 8 November 1988; accepted: 18 April 1989)

The influence of the pasteurization time and pasteurization temperature on the rheological and sensory properties of pasteurized gazpacho was studied. Gazpacho is a mixture of tomato (86.0%), olive oil (9.4%), vinegar (2.2%), salt (1.6%) and garlic (0.8).

Increasing pasteurization times and temperatures resulted decreasing yield stress and consistence index and increasing flow-behaviour index.

The values of a time-dependent parameter which reflects the structural destruction rate were smaller for the pasteurized product than for the fresh product. This parameter decreased with increasing processing time, but remained practically constant with the temperature.

Sensory tests aimed at comparing the colour, odour, flavour and texture of the products, showed that the products which differed most from the fresh product were those that had been pasteurized for the longest time and at the highest temperatures.

The agreement between the rheological and sensory results confirms the possibility of using rheological parameters as quality indices.

Keywords: rheology, sensory properties, pasteurization, food mixture, gazpacho

Gazpacho may have diverse composition. This gives rise to different types that are named after their native regions or after their most characteristic ingredient. The gazpacho studied here was "tomato gazpacho", namely a mixture of tomato, olive oil, vinegar, salt and garlic.

One simple method of preserving gazpacho could be that of pasteurization which, in contrast to freezing, does not require the use of much energy. Pasteurization also requires less investments and has lower operation costs than the other preservation method (freeze-drying).

In this work we studied the influence of the operating conditions (time and temperature) on the rheological and sensory properties of pasteurized gazpacho with the aim of finding a potential relationship between the rheological and sensory results with a view of using rheological parameters as quality indices.

1. Material and methods

A gazpacho with the following average composition in weight % was studied: tomato (86.0%), olive oil (9.4%), vinegar (2.2%), salt (1.6%), garlic (0.8%).

The total solids content of the gazpacho was 4.4% and its solid particles were less than 0.1 mm in diameter.

The pasteurizer used consisted of two heat exchangers: in the firs tthe product is heated, and in the second the product is cooled (always at 25 °C for 5 s).

So that the possible effect of shearing, which could occur when the product passes through the heat exchanger, equally affected the fresh and pasteurized products, both the pasteurized product as well as the fresh product are passed through the pasteurizer, maintaining the pasteurizer at a temperature of 25 °C.

The viscometer used consisted of a concentric rotor (35 mm diameter by 65 mm height) with a cup (38 mm diameter). The cylinder could be rotated at 8 different speeds. A more detailed description of the viscometer and its operation was given elsewhere (JIMENEZ & LOPEZ, 1983).

The sensory properties were evaluated by a panel of tasters who compared the processed samples with the fresh ones. The panelists were acquainted with the properties of the fresh gazpacho. They were selected on the criterion that in 10 sensory tests, previous to the experimental work, a 90% accuracy rate was achieved.

In this work two types of tests were carried out; one involved comparing each of the pasteurized products with the fresh one, while the other involved classifying the products in order (1 to 6) of decreasing acceptability, as compared to the fresh product (number 1).

2. Results and discussion

Gazpacho at 25 °C is used. Five gazpacho samples were heated for different times (in s) and at different temperatures (in °C): PG (θ, T). The θ denotes time and the T denotes temperature. Thus, PG (30, 70) represents a product that has been heated from 25 to 70 °C during 30 s.

Figures 1 and 2 show the plot of the first experiments, of the shear stress (τ in Pa) versus shear rate (Y in s^{-1}) for the fresh gazpacho and pasteurized gazpachos, at 20 °C, in the successive outward and return runs (increase — 3 min — and decrease — 3 min — respectively, of the viscometer speed of rotation).

The hysteresis cycles, shown in Figs. 1 and 2, were closed when $Y = 163 s^{-1}$

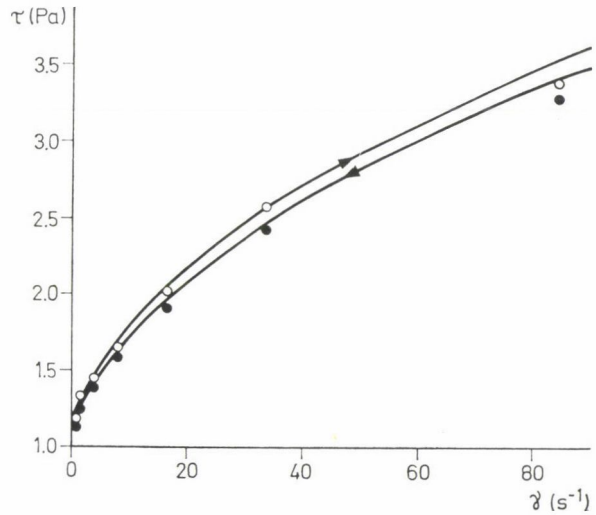


Fig. 1. Plot of shear stress (τ, Pa) versus shear rate (Y, s^{-1}) for fresh gazpacho (FG) and pasteurized gazpacho [PG, (30, 70) and PG (90, 70)], measured at 20 °C ○: Forward run ●: Return run

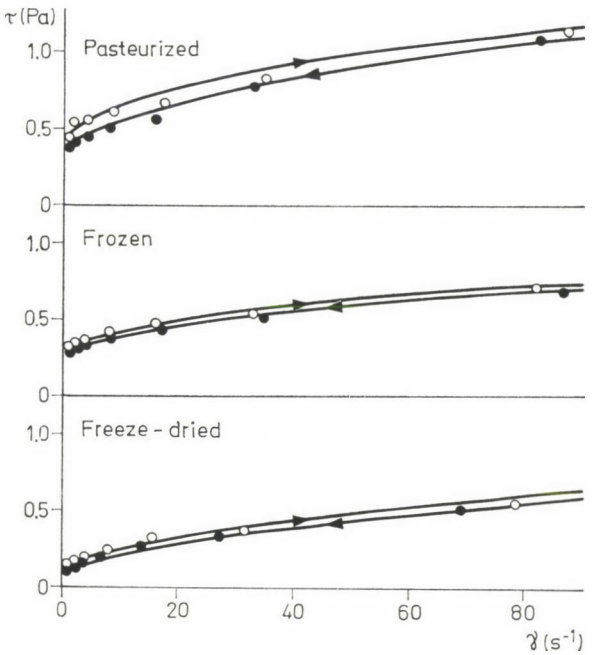


Fig. 2. Plot of shear stress (τ, Pa) versus shear rate (Y, s^{-1}) for pasteurized gazpacho [PG (10, 100), PG (30, 100) and PG (90, 100)], measured at 20 °C ○: Forward run ●: Return run

and the values of τ were 3.90, 2.72, 2.62, 2.82, 2.72 and 2.77 Pa, for fresh, PG (30, 70), PG (90, 70), PG (10, 100), PG (30, 100) and PG (90, 100), respectively.

Each product was subjected to another three similar experiments with a finding of data similar to those from before (experiments 2, 3 and 4).

As can be seen from the curves, all the products are qualitatively pseudoplastic and have a yield stress and a thixotropic time dependence.

For the rheological characterization the Herschel and Bulkley model was used (JIMENEZ & DIAZ, 1986):

$$\tau = \tau_0 + KY^n \tag{1}$$

where τ is the shear stress, Y the shear rate, τ_0 the yield stress, K the consistency index, and n the flow behavior index.

The value of the yield stress is obtained by applying the modified Casson equation:

$$\tau^{0.5}(1 + S) = 2\,(\tau_0)^{0.5} + K_0(1 + S)Y^{0.5} \tag{2}$$

where S is the relation between the radii of the rotor and the viscometer vessel, and K_0 is a characteristic constant.

Table 1

Yield stress (τ_0), *consistency index* (K) *and flow-behaviour index* (n) *of fresh and pasteurized gazpachos*

Experiment	Gazpacho	Outward run			Return run		
		10^{-2}	$K\ 10^{-2}$	n	10^{-2}	$K\ 10^{-2}$	n
1	Fresh	71.95	16.74	0.54	60.55	20.21	0.53
	PG (30, 70)	47.43	12.56	0.56	41.58	13.70	0.54
	PG (90, 70)	40.80	10.08	0.62	39.60	12.13	0.58
	PG (10, 100)	48.88	13.16	0.59	43.10	14.13	0.56
	PG (30, 100)	44,49	11.26	0.62	41.50	12.57	0.57
	PG (90, 100)	41.83	11.00	0.65	37.15	11.75	0.60
2	Fresh	73.61	15.69	0.55	58.91	21.17	0.53
	PG (30, 70)	48.39	11.65	0.57	42.49	14.52	0.55
	PG (90, 70)	40.65	9.62	0.63	39.05	11.25	0.59
	PG (10, 100)	47.95	13.95	0.58	44.17	14.98	0.55
	PG (30, 100)	44.03	12.03	0.61	41.93	13.46	0.58
	PG (90, 100)	41.53	10.15	0.65	38.03	11.05	0.62
3	Fresh	69.59	17.70	0.53	62.13	19.32	0.54
	PG (30, 70)	47.13	12.64	0.58	42.53	14.60	0.55
	PG (90, 70)	39.83	10.19	0.63	38.76	11.19	0.57
	PG (10, 100)	48.18	14.08	0.58	44.00	15.06	0.54
	PG (30, 100)	43.70	12.20	0.61	42.37	13.51	0.58
	PG (90, 100)	40.95	10.22	0.66	37.50	10.83	0.62
4	Fresh	72.15	16.05	0.53	59.81	20.80	0.52
	PG (30, 70)	46.53	13.47	0.59	42.15	14.05	0.53
	PG (90, 70)	41.78	10.87	0.62	40.57	11.31	0.57
	PG (10, 100)	49.60	13.99	0.57	44.09	14.87	0.55
	PG (30, 100)	45.45	12.13	0.61	40.59	13.39	0.56
	PG (90, 100)	42.72	10.02	0.66	38.13	10.97	0.61

Fitting the experimental τ and Y data (Figs. 1 and 2) to these equations yielded the τ_0, K and n values listed in Table 1. The correlation coefficients were always 0.99.

By operating in the same manner with the data from experiments 2, 3 and 4, the results found in Table 1 were obtained.

As can be seen from the Table, the yield stress and the consistency index are lower and the flow behaviour index is higher for the pasteurized products.

Applying the data from this Table to analysis of a variance, significant differences are found for all the products, at a probability level of 1%.

Applying the data from Table 1 for every two products to an analysis of variance, the results of Table 2 are obtained. From this table the following can be deduced:

Table 2

Results of analysis of variance for the constants of the Herschel and Bulkley equation for every two products

Compared products	Constants of Herschel and Bulkley equation					
	Outward run			Return run		
	τ_0	K	n	τ_0	K	n
Fresh and PG (30, 70)	**	**	**	**	**	
Fresh and PG (90, 70)	**	**	**	**	**	**
Fresh and PG (10, 100)	**	**	**	**	**	
Fresh and PG (30, 100)	**	**	**	**	**	**
Fresh and PG (90, 100)	**	**	**	**	**	**
PG (30, 70) and PG (90, 70)	**	**	**	**	**	**
PG (30, 70) and PG (10, 100)						
PG (30, 70) and PG (30, 100)			**			**
PG (30, 70) and PG (90, 100)	**	**	**	**	**	**
PG (90, 70) and PG (10, 100)	**	**	**	**	**	**
PG (90, 70) and PG (30, 100)	**	**		**	**	
PG (90, 70) and PG (90, 100)			**			**
PG (10, 100) and PG (30, 100)	**	**	**	**	**	**
PG (10, 100) and PG (90, 100)	**	**	**	**	**	**
PG (30, 100) and PG (90, 100)	**	**	**	**	**	**

** Highly significant at $P \geq 1\%$ probability level

— Pasteurization influences the rheology of the pasteurized products, since all of them are different from that of the fresh product.

— Upon comparing the pasteurized products during equal pasteurization time and distinct pasteurization temperatures [PG (30, 70) and PG (30, 100); and PG (90, 70) and PG (90, 100)] it is observed that the values of τ_0 and K do not differ, while the value of n increases with the increase of the pasteurization temperature.

— Upon comparing the pasteurized products at the same pasteurization temperature, but with different pasteurization time [PG (30, 70) and PG (90, 70); PG (10, 100) and PG (30, 100); PG (30, 100) and PG (90, 100); and PG (10, 100) and PG (90, 100) it is observed that the values of τ_0 and K decrease, while the values of n increase with the increase of pasteurization time.

Simulation of the experimental results of shear stress by applying the Herschel and Bulkley equation, for the first experiments, showed that the results were reproduced with deviations below 10% at worst. The variation of τ as a function of Y is shown in Figs. 1 and 2 by a continuous line.

Figures 3 and 4 display the variation of the first experiments of experimental shear stress as a function of time, at 32.5 s^{-1} and 20 °C, for all the

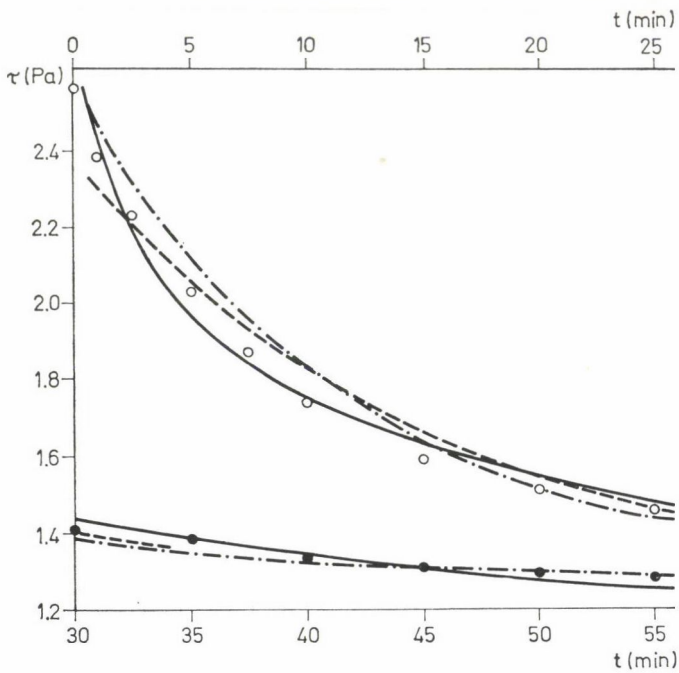


Fig. 3. Plot of shear (τ, Pa) versus time (t, min) for fresh gazpacho (FG) and pasteurized gazpacho [PG (30, 70)], measured at 20 r.p.m. and 20 °C. —: Weltman, ---: Equation 4, -.-.-: Equation 5, ○: Upper axis, ●: Under axis

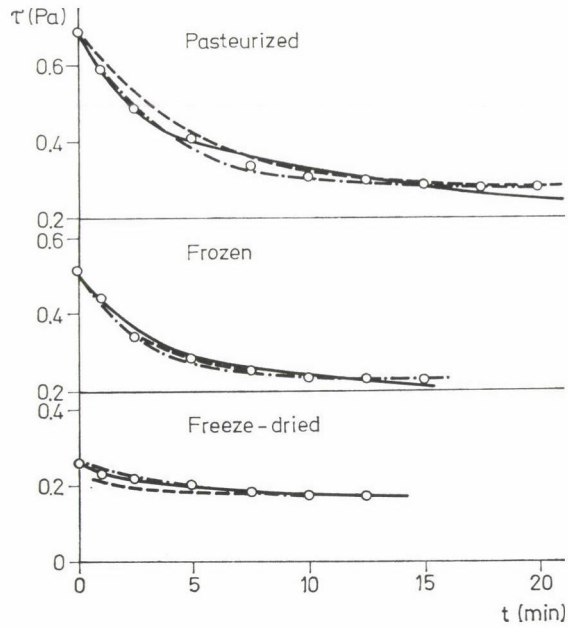


Fig. 4. Plot of shear stress (τ, Pa) versus time (t, min) for pasteurized gazpacho [PG (90, 70), PG (10, 100), PG (30, 100) and PG (90, 100)], measured at 20 r.p.m. and 20 °C. —: Weltman, --: Equation 4, ·····: Equation 5

products. Three similar experiments were carried out on each product, with a finding of data similar to those from before (experiments 2, 3 and 4).

From a qualitative point of view these products are time-dependent, and thixotropic.

In order to quantify this time dependence, we used a model assuming the product to have a complex structure, A, reversibly evolving to a simpler structure, B, through shearing:

$$A \rightleftharpoons B$$

Thus, the rate of disappearance of A can be expressed as:

$$dC_A/dt = -K_1(C_A)^{m'}f(Y) \quad (3)$$

where C_A is the concentration or fraction of A, K_1 is the rate constant of disappearance of A, m' is the order in C_A and $f(Y)$ is a function of the shearing rate.

On the other hand, the shear stress required to give rise to a given shear rate depends on the fraction of A and on the shear rate itself:

$$\tau_A = K_2X_Af'(Y) \quad (4)$$

A suitable combination of equations 3 and 4 yields:

$$d\tau_A/dt = -K_1 f(Y)\tau^{m'}/[K_2 f'(Y)]^{m'-1} = -C\tau^{m'} \quad (5)$$

where C can be considered a rate constant related to the destruction of the product structure.

As B can in turn yield A,

$$d\tau_B/dt = K(\tau_i - \tau)^{m'} \quad (6)$$

where K_3 is the rate constant of recombination of B and the shear stress at time zero.

By adding up equations 5 an 6 and rearranging, we obtain:

$$d\tau/dt = d\tau_A/dt + d\tau_B/dt = C[(\tau_e/(\tau_i - \tau_e)/^{m'}(\tau_i - \tau)^{m'} - \tau^{m'}] \quad (7)$$

where τ_e is the shear stress at time ∞, being that $d\tau/dt = 0$.

If $m' = 1$, the integration of equation 7 between τ_i and τ for $t = 0$ and $t = t$ yields:

$$\ln[(\tau_i - \tau_e)/(\tau - \tau_e)] = C\tau_i t/(\tau_i - \tau_e) \quad (8)$$

Upon fitting the experimental data of shear stress and time (Figs. 3 and 4) to equation (8), the values of C in the Table 3, were obtained.

By operating in the same manner with data from experiments 2, 3 and 4, the results found in Table 3 were obtained.

As can be seen, the C values were lower for the pasteurized products.

Realizing an analysis of variance with the data from Table 3, it is found that there exist significant differences for all the products, at a probabiity level of 1%.

Comparing every two products there are found to exist significant differences in all cases except when the following are compared: PG (30, 70) and PG (30, 100); and PG (90, 70) and PG (90, 100), that is to say the products pasteurized during equal time, but at different temperatures.

Table 3

Values of C *of equation (8) for fresh and pasteurized gazpachos*

Gazpacho	$C\ 10^{-3}$			
	Experiment			
	1	2	3	4
FG	77.70	75.80	79.83	76.19
PG (30, 70)	31.98	30.02	32.81	31.50
PG (90, 70)	25.80	25.62	24.95	26.75
PG (10, 100)	35.65	35.09	36.59	34.61
PG (30, 100)	32.94	31.81	31.98	32.32
PG (90, 100)	27.01	27.52	27.93	26.12

Therefore, the pasteurization temperature does not influence the value of the constant C, but upon increasing the pasteurization time, the value of C decreases.

Finally, upon simulation of the results obtained from equation 8, for the first experiments, it was found that they can be reproduced with deviations less than 5% for all the products assayed. The τ values, which were calculated from the aforesaid equation, are plotted in Figs. 3 and 4 as a function of Y.

In Table 4 are listed the experimental results of the sensory tests; each of the pasteurized products was compared with the fresh product.

The results obtained on comparing the fresh and pasteurized products are listed in Table 5.

Upon application of the Kramer and Twigg tables (COSTELL & DURÁN, 1987) to the data in Table 4, the results given in the same table were obtained, indicating that the characteristics of some pasteurized products are significantly different from those of the fresh product, at a probability level of 5%.

Examination of these results reveals that the products PG (30, 70) and PG (10, 100) (pasteurized at the lowest temperature and for the shortest processing time, respectively) were those yielding the smallest differences with the fresh product.

When all the products are compared simultaneously (Table 5) it is seen that there are significant differences in all the properties at the 5% probability level. Table 6 gives the order of quality of the products for all the properties. The numbers in brackets indicate the sums of the points given by the panel members for each property to the different products.

As can be seen, the greatest differences between the fresh and pasteurized products appeared in the colour; that of product PG (10, 100) was closest to the colour of the fresh product, while that of PG (90, 100) was the most different. Regarding odour, flavour and texture, products PG (30, 70), PG (90, 70) and PG (10, 100) were similar to the fresh product, while products PG (90, 100) and PG (30, 100) were the most different.

Table 4

Number of panelists who found the pasteurized products to have better properties than the fresh product

Product	Property			
	Colour	Odour	Flavour	Texture
PG (30, 70)	1*	4	4	5
PG (90, 70)	0*	3*	3*	3*
PG (10, 100)	1*	5	4	5
PG (30, 100)	2*	3*	3*	3*
PG (90, 100)	0*	3*	2*	2*

Total number of panelists = 13
* Significantly differ at $P \geq 5\%$ probability level

Table 5

Classifications made by the panelists for the fresh and pasteurized products

Property	Product	Number of panelists												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Colour	Fresh	1	2	1	1	1	1	1	1	1	1	1	1	1
	PG (30, 70)	3	2	3	3	3	4	3	4	4	3	3	4	4
	PG (90, 70)	5	5	5	4	5	3	4	5	5	2	5	5	5
	PG (10, 100)	4	1	4	2	4	2	2	2	2	4	4	2	2
	PG (30, 100)	2	4	2	5	2	5	5	3	3	5	2	3	3
	PG (90, 100)	6	6	6	6	6	6	6	6	6	6	6	6	6
Odour	Fresh	2	1	4	6	1	2	1	4	3	1	1	3	4
	PG (30, 70)	1	4	1	2	2	5	4	2	6	2	2	2	2
	PG (90, 70)	4	2	3	3	3	1	2	3	4	3	3	4	3
	PG (10, 100)	3	6	2	1	5	3	6	1	1	6	6	1	1
	PG (30, 100)	5	5	5	4	4	4	3	6	5	5	5	6	5
	PG (90, 100)	6	3	6	5	6	6	5	5	2	4	4	5	6
Flavour	Fresh	1	4	6	1	2	2	1	1	2	2	2	1	5
	PG (30, 70)	3	2	1	2	1	4	3	2	3	6	3	2	2
	PG (90, 70)	5	3	2	5	6	1	5	5	4	1	4	6	3
	PG (10, 100)	2	5	4	3	3	3	2	3	1	4	1	3	4
	PG (30, 100)	6	1	3	6	4	5	6	6	6	3	6	5	1
	PG (90, 100)	4	6	5	4	5	6	4	4	5	5	5	4	6
Texture	Fresh	2	3	2	3	3	3	5	1	4	3	1	3	2
	PG (30, 70)	1	5	6	1	5	1	2	3	2	2	3	4	1
	PG (90, 70)	4	2	5	2	1	2	3	2	5	4	2	2	5
	PG (10, 100)	3	4	1	6	4	4	1	5	1	1	5	6	3
	PG (30, 100)	5	1	3	4	2	6	6	4	6	5	4	5	6
	PG (90, 100)	6	6	4	5	6	5	4	6	3	6	6	1	4

Score: 1 best, 6 worst, respectively

Table 6

Overall classification of the products, in order of decreasing quality

Order of quality	Characteristic			
	Colour	Odour	Flavour	Texture
1	Fresh (15)	Fresh (33)	Fresh (30)	Fresh (35)
2	PG (10, 100) (35)	PG (30, 70) (35)	PG (30, 70) (34)	PG (90, 70) (36)
3	PG (30, 70) (43)	PG (90, 70) (38)	PG (10, 100) (38)	PG (30, 70) (39)
4	PG (30, 100) (44)	PG (10, 100) (42)	PG (90, 70) (50)	PG (10, 100) (44)
5	PG (90, 70) (58)	PG (30, 100) (62)	PG (30, 100) (58)	PG (30, 100) (57)
6	PG (90, 100) (78)	PG (90, 100) (63)	PG (90, 100) (63)	PG (90, 100) (62)

Table 7

Global quality scale for the fresh and pasteurized products

Product	Total score	Reduced score[a]	Order of quality
FG	113	1	1
PG (30, 70)	151	1.34	2
PG (10, 100)	159	1.41	3
PG (90, 70)	182	1.61	4
PG (30, 100)	221	1.96	5
PG (90, 100)	266	2.35	6

[a] Reduced score: Total score of each product per total score of fresh product

By means of a global approximation, considering the four characteristics studied, the quality scale for the different products could be obtained by summing-up the scores of the different characteristics for each product. The global quality scale is given in Table 7.

The reduced scores were obtained by dividing the total score of each product by that of the fresh product.

3. Conclusions

The pasteurized products have lower yield stress, consistency index and structural destruction rate constant, C, than the fresh product. However, the flow-behaviour index was greater for the pasteurized products.

Upon increasing the pasteurization time, the products present lower values for τ_0 and become less consistent, depending upon the time and pseudoplastics.

Upon increasing the pasteurization temperature in the tested interval (70–100 °C) the products maintain their value for τ_0, and do not change their consistency and dependency on time, but become less pseudoplastic.

Regarding quality, products PG (30, 70) and PG (10, 100) were confirmed to be those with the most desirable properties, while PG (90, 100) and PG (30, 100) were found to be the worst ones as compared with the fresh product.

Comparing the results of the rheological analysis and those of the sensory tests, the conclusion can be reached that the values of the rheological parameters can be used as quality indices for the pasteurized products: the lower the values of τ_0, K and C, and the more elevated the value of n the less acceptable the quality of the pasteurized gazpachos.

Literature

COSTELL, E. & DURAN, L. (1987): El análisis sensorial en el control de calidad de los alimentos. IV. Realización y análisis de los datos. *Revta Agroquim. Tecnol. Alimentos*, *22*, 1–21.

JIMENEZ, L. & DIAZ, R. M. (1986): Estudio reológico de los componentes de la mezcla aceite de oliva-huevo-zumo de tomate. *Afinidad*, *43*, 311–320.

JIMENEZ, L. & LOPEZ, A. (1983): Viscosímetro rotatorio para medidas reológicas de productos líquidos. *Afinidad*, *40*, 448–452.

Acta Alimentaria, Vol. 19 (2), pp. 199–213 (1990)

30th ANNIVERSARY OF THE CENTRAL FOOD RESEARCH INSTITUTE, BUDAPEST, 1989

ABSTRACTS

of papers presented at the Scientific Session held at the

CENTRAL FOOD RESEARCH INSTITUTE

on June 15 1989

ADDRESS OF WELCOME

J. Holló

chairman of the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food, Budapest, Hungary

Hungarian food science originates in ancient times: just think of the meat of nomad Hungarians made tender under their saddles, koumiss prepared from mare milk, or Tokay Aszu, which became world-famous already in the Middle Ages. In some of our products, a characteristic "Hungaricum" flavour and aroma have been developed, the improvement of which is set as an objective by one of our present economic government programmes, as well. Let me mention here, among others, salami, apricot brandy, spiced sheep-cheese (the so-called "körözött"), Dobos cake, pastry horn or hot paprika. Hungarian technology also largely contributed to rendering our food industry world-famous, just like Ganz-Mechwart's high grinding, Haggenmacher's plainsifter or Hankóczy's farinometer; in the history of starch production, there is a so-called Hungarian technology mentioned, too, which differs from the up-to-date wheat starch production only in so far as its starting material is whole grain instead of wheat flour.

In my opinion, the conscious development of Hungarian food economy was considerably promoted by the fact that over a century ago Ignác Darányi, minister of agriculture of that time, established the agricultural research network. Let me note here in brackets that a neighbouring street was named after him until the fifties. In the era of rehabilitations, so highly popular in Hungary today, we have every right to suggest: another street in the area should be named after Ignác Darányi. This experimental network had several research branches, such as department for food qualification, or a department dealing with the technological changes of food products. Vince Wartha, the first professor of Chemical Technology at the Faculty of Chemical Engineering, Techni-

cal University, Budapest founded in 1872, dealt with problems of the food industry systematically, too. A new impulse was given our profession, both by their achievements in training and research by the Department of Agricultural Chemical Technology established in 1908 and the Department of Food Chemistry founded in the early twenties.

As a matter of curiosity, from the twenties on, acetonebutanol fermentation gained ground in our country. From 1928 it was made obligatory to mix anhydrous alcohol into petrol, and during the Second World War, glycerine fermentation was also realized in a Hungarian factory. Thus, Hungarian food industry can be considered very expansive and successful until the Liberation of the country.

Research came to life again very quickly over the ruins and an even greater momentum was given to its development by the creation of state food industry. Small company-size experimental laboratories and state research centres gradually gave place to different industrial research institutes, which have been, since then, greatly contributing to keep Hungarian food industry and not least food science on an international level.

In the mid-fifties it was raised, as an expedient idea, to establish — beside industrial research institutes — a centre, which would deal with more general problems of food science, in the chemical, biological, operative, mechanical engineering and, last but not least, economic fields. On this idea was the Central Food Research Institute created 30 years ago.

The first director of the Institute was Prof. Gábor Török, who was a great scholar of a wide intellectual horizon, an inventive chemical engineer, similar to whom I hardly ever knew. He was a matter-of-fact man who could think systematically in drawing the consequences and immediately utilizing the latest scientific achievements, and last but not least, a man of sparkling wit and profound humour; he was a really great man. He was good at choosing his colleagues, too; many a young — today leading — researcher and production engineer owe their career to him. I myself, was also on good friendly terms with Prof. Gábor Török; from the very beginning he enthusiastically supported the activities of our scientific society, and, walking home from our presidential meetings, he always gave me friendly pieces of advice helping me to solve my everyday scientific and technical problems. He was the one who started almost every research line at the Central Food Research Institute which deservedly rendered this Institute renowned. Prof. Károly Vas, member of the Academy, who createp Hungarian food microbiology and who was the first to apply food irradiation preservation, started from this place towards world reputation, just like Dr. Pál Spanyár laid here the foundations for modern food analytics, and Prof. Elemér Almási, founder of Hungarian research in the field of refrigeration and deep-freezing, also worked here for some decades. On this occasion we wish to render homage to them, as well.

On behalf of the Complex Committee for Food Science of the Hungarian Academy of Sciences and the Presidium of the Hungarian Scientific Society for Food Industry, I am both pleased and honoured to congratulate you on this anniversary. I wish you to be able to celebrate many more successful 30 years, since this is one of the most promising pulling branches of our national economy !

FOOD IRRADIATION — STATUS AND PROSPECTS

J. F. DIEHL

Federal Research Center for Nutrition, Karlsruhe. FRG

Close to 20 000 tons of food are irradiated annually in The Netherlands, over 10 000 t in Belgium, and over 5000 t in France. Food irradiation on a similar scale is carried out in the Soviet Union, the People's Republic of China, South Africa and Japan. Smaller quantities, though with an upward tendency, are irradiated in the German Democratic Republic, Brazil, Israel, Thailand, Hungary and some other countries. In contrast, a vigorous public opposition still prevents the introduction of this process in countries like the United Kingdom, Australia and the Federal Republic of Germany. In the United States, irradiation of spices is carried out on a small scale and the opposition has prevented a more widespread application. This opposition is primarily based on the claim that the safety of irradiated foods has not been proven. A critical analysis of arguments directed against food irradiation shows that they are not based on facts. The conclusion of the 1980 FAO/IAEA/WHO Joint Expert Committee (JECFI) that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard, still stands. This conclusion was based on a massive international research effort, some of which was carried out at KÉKI under the able leadership of Professor Károly Vas. No results or arguments presented since then have given valid cause to contradict this conclusion. On the contrary, the results of studies carried out with food irradiated at much higher dose levels have shown that JECFI's approach was conservative.

If the safety of irradiated foods has become a subject of heated debates in the European Parliament and in many national legislatures it is for political reasons. The antinuclear movement has gained ground in recent years, particularly since the Chernobyl disaster, and to many representatives of that movement food irradiation is part of the "plutonium economy". Fabricated accusations implying that the nuclear industry has invented food irradiation as a means of getting rid of their nuclear waste have been too readily accepted by some consumer organizations and representatives of the media. In fact, wherever food irradiation is now carried out on a commercial scale, this is done

with gamma radiation from cobalt 60-sources or with electron radiation from electron accelerators. Neither one has anything to do with nuclear waste. Cesium-137 is a nuclear waste product and there have been suggestions for using it in food irradiation. However, these suggestions have not led to practical applications anywhere, and it appears quite unlikely that this isotope will play a significant role in food irradiation in the future. The claimed association of food irradiation with the "plutonium economy" is totally unjustified.

Arguments against food irradiation come not only from the anti-nuclear camp but also from those who favour natural, biological, unprocessed foods. Primarily from this group comes the demand that "absolute safety" of irradiated foods must be proven before the process is permitted. It is of course impossible to prove absolute safety. In a recent bulletin WHO has stated: "Widespread information campaigns are still required for food irradiation to be fully accepted. WHO is concerned that rejection of the process, essentially based on emotional or ideological influences, may hamper its use in those countries which may benefit the most." Those who have been active in the field of food irradiation for many years and who still believe that this process could play a useful role in preventing food-borne disease and reducing food losses, share this concern.

EVALUATION AND INTERPRETATION OF COLOUR MEASUREMENT DATA OF FOODSTUFFS

A. Schaller

Institut für Lebensmitteltechnologie, Universität für Bodenkultur, Wien. Österreich

First of all, the three-dimensional and approximately uniform a^*, b^*, L^* colour space (Commission Internationale de l'Éclairage, 1976) has been discussed with the help of some graphical illustrations (Rectangular coordinates a^*, b^* and L^*/Colour difference ΔE^*_{ab}/Components of ΔE^*_{ab}: ΔL^*, Lightness difference, ΔC^*_{ab}, Chroma difference, and ΔH^*_{ab}, Hue difference (Sign of ΔH^*_{ab} and its meaning).

Following this introduction, the application of the a^*, b^*, L^* colour space has been demonstrated with the evaluation and interpretation of the colour changes of deepfrozen "cold-break" tomato pulp during a 0.5 a storage at 253 K (−20 °C). The colour measurements in the course of storage time were done at 293 K (+ 20 °C) using a tristimulus colorimeter Momcolor D (Magyar Optikai Művek, Budapest) under the following conditions: 0/45 °C optical geometry, observing field of 2°, standard illuminant C, white enamel standard (Országos Mérésügyi Hivatal, Budapest), 50 mm layer-thickness of tomato pulp in glass cuvette, etc. At each of 8 storage times (1 to 182 days) 5 samples of

thawed product were measured. The estimated mean values of a^*, b^*, and L^* have been used to calculate the derived quantities (Δa^*, Δb^*, ΔL^*, ΔE^*_{ab}, ΔC^*_{ab}, ΔH^*_{ab}) whereby the values at the beginning of the frozen storage (at day 1) served as the references. As could be seen from the presented graphical illustrations, the statistical evaluation of the colour measurement data yielded the following results.

There existed non-linear, highly significant ($P < 0.001$) decrease of Δa^* with increasing storage time ($100\ r^2 = 99.2$) a non-linear, highly significant ($P < 0.001$) increase of Δb^* ($100\ r^2 = 98.6$) and a non-linear, highly significant ($P < 0.001$) increase of ΔL^* ($100\ r^2 = 99.0$).

The connection between the colour difference (ΔE^*_{ab}) and the storage time was non-linear, highly significant ($P < 0.001$) and very close ($100\ r^2 = 99.8$). Splitting the colour difference in its components and statistical evaluation of the data provided the following informations of special interest.

ΔC^*_{ab} showed a non-linear, highly significant ($P < 0.001$) decrease with increasing storage time ($100\ r^2 = 95.3$) and in the case of ΔH^*_{ab} a non-linear, highly significant ($P < 0.001$) increase existed ($100\ r^2 = 99.5$).

Therefore, the increase of the colour difference with increasing storage time of the frozen "cold-break" tomato pulp was caused by the increase of lightness difference as well as by the increase of hue difference whereby the contribution of the latter was more marked. In contrast, the chroma difference decreased with increasing storage time, especially in the first six weeks of frozen storage.

In respect to the importance of hue difference for the colour changes observed, the hue-angles — $h_{ab} = \arctan(b^*/a^*)$ — were statistically evaluated. There existed a linear, highly significant ($P < 0.001$) increase of about 35 to 53° with increasing storage time ($100\ r^2 = 99.6$). Thus, the positive sign of the hue-angle difference as well as of the hue difference showed that the hue of the product was changing towards yellow (90°). These time-dependent colour changes are caused by degradation reactions of the natural carotenoid pigments as lycopene and others (see Gy. Urbányi & K. Horti, Hűtőipar, Vol. 35 (1989), No. 1, 19–24).

With respect to the frequent use of the quantity a/b (a, b, L colour space after R. S. Hunter) in the routine colour control of tomato products, the data of the quantity a^*/b^* have been also statistically evaluated. A linear, highly significant ($P < 0.001$) and very close ($100\ r^2 = 98.2$) decrease (from 1.43 to 0.75) with increasing storage time has been found. In our opinion, the quantity a^*/b^* is inefficient to characterize the colour or colour changes of tomato products.

PLANT BREEDING AND FOOD PROCESSING

A. RUTKOWSKI

Warsaw Agricultural Unversity, Warsaw. Poland

A few years ago, the main goal of food industry was the profit obtained by the utilization of agricultural crop. At present more and more agriculture is recognized as initial stage of food production and is directed by consumers. This is a consequence of increasing consumption of industrially processed food. Quite naturally the farmers' criteria in breeding were directed toward yields. From the viewpoint of the processor and the consumers the main objectives in selection should be concerned, either directly or indirectly with the processing properties of the crop and the nutritional value of product. Therefore the improvement of quality is thus an important objective that plant breeders are starting to set as a target. Similarly important is the study on the improvement of processing technology in the industry. Rapeseeds is a good example of this changes and genetic and technological intervention in this field. I have in mind first of all:

— increase of the crop of seeds, as well as of oil protein content in seeds;
— drastic reduction of erucic acid and diminution of linolenic acid in oil;
— reduction of glucosinolates and decrease of fiber content in the meal.

F a t t y a c i d s. Laboratory animals fed on high erucic acid containing rapeseed oil (HEAR) show pathological symptoms in the heart muscle and in consequence the oil was considered as a danger for human health. The risk factor has been identified as erucic acid (C 22 : 1 w 13) representing 50% of total. Removal of this acid from glycerides by a technical method is impossible. Fortunately plant breeders found a natural mutant stopping the biosynthesis of mono-unsaturated fatty acids at the level of oleic acid. This enabled complete change in the fatty acid composition of oil and low erucic (less than 1%) and high oileic acid containing (C 18 : 1 w 9) rapeseed oil (LEAR) was obtained. In this oil the proportion of 18-carbon atom fatty acids amounts to 90%. What are the technological consequences of this fact?

1. The higher degree of oxidation reactivity of unsaturated fatty acids of LEAR cause relatively lower resistance coefficient to oxidation and amounts to a LEAR : HEAR ratio of 0.8 : 1. This is also expressed by the greater readiness to form polar products or polymerised triglycerides when LEAR is used for frying.

2. Different structure of triglycerides cause that the melting curve of HEAR is narrow and attains its maximum at +2 °C. On the other hand, LEAR covers a much broader range ending at −13 °C.

3. The higher reactivity of oleic acid with hydrogen than that of erucic acid cause that the hydrogenation of LEAR oil is more violent with a stronger exothermic reaction. Moreover only a small quantity of erucic acid (up to 4%) of HEAR is hydrogenated to behenic acid while the greater part undergoes trans-isomerization. LEAR hydrogenated to the same IV-oil, is ofter than HEAR and consists of lower solid glycerides content with a higher melting point.

4. The hydrogenated LEAR oil, like other high-oleic acid containing fats tends to recrystallize its beta prim crystals to beta crystals that are responsible for the grainy, sandy structure of margarine. This phenomenon, may be counteracted by the increase of palmitic acid content in the formula.

S u l f u r is the second specific component of rapeseed and is present up to 1.5%. Most of the sulfur (about 2/3) occurs in the form of glucosinolates. Sulfur and its compounds hinder the hydrogenation process by contaminating the catalyst and speed up the corrosion of processing equipments. Glucosinolates disturb the utilization of rapeseed meal as fodder for monogastric animals. The following consequences of sulfur compounds in rapeseed have to be drawn:

1. Rapeseed oil used for hydrogenation needs careful refining to remove sulfur compounds.

2. Thermal processing (toasting) of rapesed meal should be used for the reduction of glucosinolates content for improving the nutritive value of rapeseed meal.

3. Reduction by breeding of glucosinolates content in repaseed to 10% of the original by low glucosinolate varieties improved considerably the quality of meal and reduced difficulties in the course of hydrogenation of the oil. Nutritive value of this meal is comparable to that of soybean meal.

4. The reduction of the fiber content in rapeseed meal by hulling of seeds improved the quality of proteinous feeding meal.

Conclusions. Improved rapeseed varieties, beside their advantages, present novel technological problems and these have to be resolved by the technologists. To achieve a high quality product close cooperation of plant breeders and technologists is essential.

MEMORIAL SPEECH

and

Unveiling the Plaque

I. TÓTH-ZSIGA

general secretary of the Hungarian Scientific Society for Food Industry, Budapest. Hungary

I feel it a privilege to honour in the name of all those who had the good fortune to personally know or even collaborate with the great generation of our forebears founding, developing and promoting food science in Hungary.

It is well known that the tremendous development in food science started after the last war and in the past 40 years the generation of leading scientists, researchers determined the trend of development in this field not only for the present but also for generations to come.

We wish to express our respect to such great personalities of food research as Gábor Török, Kossuth-prize holder, professor and first director of our Institute, Károly Vas, professor, academician, Elemér Almási, professor, Government-prize holder and Dr. Pál Spanyár breaking fresh path in food analysis. These men have founded a school within the food industry and participated with their knowledge even under severe conditions in the development of the industry. We pay homage also to the memory of colleagues here not enumerated but well known to all of us who contributed also by their internationally acknowledged work within the Central Food Research Institute (C.F.R.I.) or other research institutes, to the appreciation of this work. Thus the Board of the Hungarian Scientific Society for Food Industry decided to award to the research team of the Central Food Research Institute the Gábor Török medal created to commemorate the great founder of the Institute. These scientists are the ones whom it becomes the quotation from the ode of Horace: Exegi monumentum aere perenniis.

We wish that the members of the Central Food Research Institute or those of other institutes would take pattern by their forebears and would follow in their footsteps.

The nation that appreciates his past deserves his future. Our appreciation should serve to encourage the present and future generations to create further imperishable results.

ABSTRACTS

of papers

presented by the staff members of the Central Food Research Institute at the Scientific Colloquia organized by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and the Hungarian Scientific Society for Food Industry and the Central Food Research Institute

RESEARCH ORGANIZATIONAL TASKS AND ACTIVITY OF THE CENTRAL FOOD RESEARCH INSTITUTE

P. Biacs, I. Varsányi and L. Somogyi

The work of the C.F.R.I. and its research organizational activity is directed by the Department for Food Industry of the Ministry of Agriculture and Food, as supervising authority, as well as the Department for Education and Research, taking into account the decisions of December, 1982 of the Presidium, of Hungarian Academy of Sciences.

Among the tasks of the C.F.R.I. an important role is assigned to the organization of research and to the information activity forming an important part of this. The research organizing activity asserted itself mainly in the organization of programmed research and the secretarial activity attached to it. In the past five years the coordination tasks at ministerial level and in the present 5-Year Plan Period the secretarial activities of the National Medium Range Research and Development Program, were performed. In these two programmes the research project development work of about 100 to 150 institutions was coordinated. The coordination activity extended over the dissemination of informations on the results through the trade journals and by exhibitions.

Collaboration with inland enterprises and institutions was extended beyond programmed research. About 100 research and development tasks at enterprise level were looked after as a result of which new products, modern technological processes and know-hows were borne.

Within the frame of socialist contracts the institute collaborated with about 30 institutions. This meant in addition to the exchange of information, useful division of labour and expedient instrument management.

Beyond organization of research at home we aimed at establishing and tending many-sided international contacts. International collaboration is realized in technical-scientific intergovernmental agreements, in the course of activities in COMECON committees and work teams, by way of expert participation and representation in international organisations and in direct contacts between institutes. The importance of this multiple activity is sustained by the fact that the leading research workers of the Institute participate, beyond their

research work in expert, advisory and tutorial activity in accordance with the agreements.

Summing up, it can be established that significant results were borne from the research organizing work carried out in accordance with the expectations of the supervising authority and the Hungarian Academy of Sciences.

INNOVATION IN THE FOOD INDUSTRY

P. Gerely

In recent years the innovation activity in the food industry increased significantly. Main fields of these activities were:

— product development,
— development of the technique applied,
— development of enterprise management.

Since 1982 in the division of investment in the food industry according to targets of research and development, no significant change occurred (product development 31%, development of technology 36%, the rest 33%). However, the investment in different branches of the individual fields differed significantly. The proportion of new products in marketing (calculated on the basis of price income) amounted to 0.3–0.5%. The number of international cooperative agreements increased three-fold between 1982 and 1987 (53 agreements in 1987), while the proportion of cooperations in the field of R + D exceeds 14%.

The logic of development and particularly of product development, based on the formation of market relations, is presented pertinent to the dairy industry as an example. The rapid rise of material and energy costs significantly stimulates the development of technology and technique. The maintenance of health is today a national program. It is regrettable that the reduction in the standard of life does not promote the large-scale consumption of these products which are anyhow generally not cheaper than the traditional ones. Processes occurring in the home economy and on foreign markets, the narrowing of the home market, the rapid growth of expenses leads to further straining of market conditions. Thus, the continuous development of production and products, the diversification of products, improvement of quality and innovative way of thinking will spread at a rate observed in recent years or at an even higher rate. Basic research as applied in recent years, we hope, will serve with the necessary scientific basis to development.

SAFE PROTEINS

É. GELENCSÉR

Food producers have to aim at safeguarding consumers' interests, at producing food proteins of standardized, good quality and safely consumable. Safe utilization depends basically on the origin and the way of processing of the goods.

The task of the Department of Food Biology of C.F.R.I. is the detection and elimination of factors bearing health hazards (e.g. allergens, biological contamination, reaction products of technological processes, etc.) and the investigation of the effect of treatment technology upon protein quality.

The aim of this paper is to prove the importance for food science and industry of biological methods through the results of research work on the improvement of the quality of food proteins. New methods to detect proteins potentially dangerous to the consumer, are discussed.

Methods suitable to characterize the quality of food proteins, to detect potentially allergy causing antigeneity and specific antibodies, are available at the Department. The methods developed at the Department render possible the detection of protein of foreign origin or the declaration of the absence of protein. Thereby the number of protein-allergic patients can be reduced and the production of non-hazardous proteins improved and the range of up-to-date proteins enlarged.

CHEMICAL AND PYHSICAL ANALYSIS OF FOODS

F. KULCSÁR, M. PETRÓ-TURZA and M. VÁRADI

Food analysis plays an important role in food control, quality control but its importance is not negligible in research into food chemistry and biochemistry.

This paper gives an account of research results achieved at the Department of Food Analysis of C.F.R.I., in the last five years.

Research into chemical analysis of foods was primarily aimed at detecting the aroma composition of raw materials and food products and at the development of pertinent analytical methods:

— A draft standard was prepared on the analysis of compounds and groups of compounds most frequently occurring in aroma preparations and on the quantitative measurement of the solvents used;

— The natural aroma components of fruits, vegetables and spices as well as the relationships between their composition and their sensory quality,

were investigated. The interrelations between the aroma substances and the non-volatile components of foods were studied, too.

Aroma research was complemented by concrete industrial tasks in which analysis served as a tool.

— A process and equipment were developed and patented to prepare natural aroma concentrates.

— The assembling of aroma compositions and ways of synthesizing individual important aroma components, suitable for their manufacture, were developed.

A further subject of research was to work out a complex method for the determination of the proportion of fruit in import raw material used to produce citrus fruit drinks. In the frame of this study the characteristics of nearly 30 components of the fruits were investigated.

In recent years out of the physical characteristics of fruits the optical properties came to the fore at the Department for Physics of C.R.F.I. The investigations were aimed at the composition of the various raw materials, semi and end products of foods and their correlation to their reflection or transmission spectra taken in the visible or near infrared wavelength range. The rapid, non-destructive measuring methods developed permit:

— the determination of the components of cereals and oil-seeds,

— the measurement of the moisture content in ground paprika, pulverized cocoa and coffee,

— the classification of milk and milk products, meat and meat products,

— the determination of the egg, protein and fat content in pasta products,

— the measurement of the edible fibre content of bran,

— the establishment of the amino acid composition in lupin,

— the establishment of the stage of maturity of stone fruit.

Most of the research results were adapted to the generally used equipment of routin laboratories and thus, they may be put into practical use.

RECENT RESULTS OF AMYLOLYTIC ENZYMES RESEARCH

Á. Hoschke

The dinamically growing utilization of amylolytic enzimes in Hungary and the increasing export possibilities to COMECON countries raised the necessity of enzyme production in this country.

The paper describes recent results of strain improvement and optimization of enzyme fermentation aimed at increasing amylolytic enzyme production at the Food Biotechnology Division of C.F.R.I.

Research into strain improvement

In order to obtain a super enzyme producing strain, meeting the conditions of economic industrial production, a multi-step combined stain improvement strategy (chemical and physical mutation, recombinant DNS technique) was developed.

To increase thermostable α-amylase production the ATCC 27811 *B. licheniformis* strain was used. A 20-fold increase in enzyme production was achieved by multi-step UV mutation and selective isolation technique. Parallel to the mutation experiments strain improvement by genetic engincering was also started. The genomic library of *B. licheniformis* was constructed and the steps of transformation were optimized.

In the course of strain improvement for glucoamylase production, in the first step 150 fungal strains were selected and qualified. For strain improvement a wild strain, isolated from corn (KÉKI P-36) and an induced mutant of *A. niger* (ATCC 22343) originating from a strain collection, were used. With the wild strain by multi-step mutagenesis the enzyme production increased about 25-fold. For the genetic improvement by genetic engineering the *A. niger* strain (ATCC 22343) was used. Several hundreds of recombined strains were isolated by protoplast fusion, however, the enzyme production of these strains did not reach that of the parent strains. In the course of the investigations based on recombined DNA technique the genomic library of the strain was brought into existence and by DNA hybridization the gene sequences responsible for glucoamylase production, were identified.

Optimization glucoamylase fermentation

The optimization experiments were carried out in shaken flask cultures and in 10-litre fermentor. In this experimental series *A. niger* (ATCC 22343/65) strain was used.

The composition of the medium was optimized in shaken flask culture according to a complete factor scheme. The changes in glucoamylase, transglucosidase and α-amylase activities, mycelium mass, pH and glucose content were measured during fermentation.

On the basis of the results of these experiments the optimization of conditions of the submersed enzyme fermentation was continued in 10-litre fermentors. Under optimized conditions 18–20 GAU cm^{-3} reproducible enzyme production was achieved. The technology thus developed permits economic realization of "in house" fermentation technique, in accordance with industrial requirements.

CHARACTERIZATION OF YEAST PROTEASES

A. Halász, M. Szakács-Dobozi, Gy. Hajós, B. Mátrai and I. Szalma-Pfeiffer

The changes in protease activity in *S. cerevisiae* baker's yeast were studied in batch fermentation during the growth phase. Further the effect of glucose concentration and aeration intensity on the specific and non-specific proteolytic activity or on protease inhibitor level, was examined.

The proteinase profile of the raw extract is significantly affected by the method of cell breakdown and the activation procedure applied as is shown unambiguously in the pH optimum as well as in the temperature optimum.

The study of specific proteinase activity (Leu-amino peptidase, carboxypeptidase S, carboxypeptidase Y, proteinase A, proteinase B) has proven that it is only the carboxypeptidase Y activity which is not affected by the aerating intensity changes, otherwise the higher the aeration intensity the lower is the proteinase activity.

It was established that the specific proteinase inhibitors (I_a and I_B) react in the same way as the corresponding proteinase to changes in glucose concentration and aerating intensity.

Preparative IEF fractions of the raw extract with specific proteinase activity were applied as antigen to develop antibody in rabbit. The immune serum, thus obtained, was applied by ELISA technique to measure changes in the concentration of enzyme protein upon growth phase, glucose concentration and aeration intensity.

The carboxypeptidase Y activity present in the raw extract of brewer's yeast catalyses successfully the incorporation of amino acids by EPM reaction. The incorporation of amino acids occurs exclusively at the C terminal.

Changes in the *S. cerevisiae* proteinase activity and protein fluorescence were studied in a glucose-limited oscillating chemostat system. Within one cycle the enzyme activity showed a leap of two orders of magnitude. Maximum enzyme activity appeared at maximum CO_2 production. Protein fluorescence oscillated in the course of one cell cycle, too.

APPLICATION OF MEMBRANE SEPARATION IN THE FOOD INDUSTRY

L. Mester

During the 30 years of the existence of the C.F.R.I. research into membrane separation brought about significant results. The great forebears, Dr. Mihály Demeczky and Mrs M. Khell realized early the possibilities inherent in

this new technique, successfully applicable in separation, concentration and cleaning processes of the food industry. Membrane filtration is an extremely mild operation, capable of maintaining the values inherent in foods and their taste and aroma substances. There are three main fields of research in relation to membrane filtration:

— filter membranes,
— membrane filter modules and equipment,
— membrane separation technologies.

This paper accounts for the results achieved, at this institute in the development of plane membrane, membrane manufacture as organised at the CFRI and the types of membrane marketed. The latest result is the development of the inserted ultrafilter spiral module, in addition to this, in cooperation with the Water-engineering Enterprise the plane disk, and with the VEGYÉPSZER enterprise the spiral module system, were developed.

Research work on the application of membrane separation in the food industry was extended over the main separation and cleaning processes of which the most important are: concentration of mineral water, processing of blood, clearing fruit juices, among them apple juice. Membrane separation was worked out for the dairy industry and also for environmental protection tasks.

BOOK REVIEWS

Safety of irradiated foods

(Food Science and Technology Series, 36)

J. F. DIEHL

Marcel Dekker, Inc., New York, 1990, 345 pp.

The status of food irradiation is characterized by the availability of a vast amount of factual scientific information and, at the same time, a deluge of public misunderstandings and controversial debates on the subject. Therefore, conveying factual information to the non-specialists and allaying consumer fears concerning the safety of irradiated foods are badly needed. Professor Diehl is not only a world authority on chemistry, nutritional and toxicological evaluation of irradiated foods but also an excellent educator. Thus, his book serves eminently this purpose.

The first chapter of the book provides a concise and well documented review on radiation technology, radiation chemistry and radiation biology of foods. Chapters 5 to 8 deal with the central issue: wholesomeness of irradiated foods regarding to their toxicological and microbiological safety, nutritional adequacy and the evaluation of these results by expert groups and specialized international organizations. The final four chapters concentrate on potential and actual applications of food irradiation, as well as on regulation and consumer acceptance. Of particular interest is the penultimate chapter which discusses arguments against food irradiation by anti-nuclear movements and some consumer advocates. This chapter provides clear advices and sensible comments on all misgivings. The book is supplemented with the reproduction of the basic Codex Alimentarius documents relevant to the subjects: The Codex General Standard for Irradiated Foods, and The Recommended International Code of Practice for the Operation of Irradiation Facilities Used for the Treatment of Foods.

This book meets the declared objectives of the author and it has a great value to individuals and organizations, who are seeking real information on food irradiation, particularly from the field of public relations and education.

J. FARKAS

Dictionary of foods and food processes

(English, German, French, Spanish, Italian)

L. RAYNER (Ed.)

Food Science Publishers Ltd, England, 1990, 290 pp.

The book has been compiled as a reference for those who need to find an equivalent word of another language for a particular food or process. It consists of over 4200 terms used within the food industry in five languages (English, German, French, Spanish and Italian).

The dictionary has not followed the usual practice of using only one language as the main method for searching, and then referring the reader elsewhere. Instead, each term is included with foreign equivalents making the book easyto use for immediate reference. An equivalent term, within the same language, is not given alongside its comparative

work and appears on its own and there is an entry for a synonymous term wthin the appropriate alphabetical section.

Varieties of certain foods are included plus well-known cheeses, wines and types of meat cuts and preparations.

The dictionary is very useful in the practical and theoretical fields, including the education and research.

I. VARSÁNYI

Electricity usage in the sugar industry: Opportunities for reducing the demand

VDZ Proceedings, CURDTS, U. and EICHHORN H. (Eds)

Verlag Dr. Albert Bartens, Berlin, 1989, 104 pp.

The book to be reported contains the complete texts of the ten lectures delivered on the General Assembly of West German Association of Sugar Technologists (VDZ) in German and English.

In the last two decades in several West-Europian countries — among them in FRG — the specific use of steam was managed to reduce to its half, at the same time the specific use of electric energy increase to a certain extent because of the introduction of new technologies, mechanization and automation. The balance of the use of electric and heat energy produced in the power plant of the sugar factory itself, however, was overturned. This overturn has a reaction on the efficiency and economicalness of the energy utilization. A many-sided discussion and some recommendations aimed at the solutions are the topics of the above mentioned book. The authors were gathered from the outstanding experts of the sugar technology, and electric energetics.

The introductory paper summarizes the development of the energetics and operation techniques as well as of the perspective of sugaring. It contains detailed quantitative figures of the energy consumption of the German food industry, too, giving a priority to the specific steam consumption related to the end products of sugar factories which decreased from 16 MJ/kg to 10 MJ/kg in the period of 1978—1985 while the specific electric energy consumption was measured at about 270 Wh/kg.

The other lectures deal with many-sided analyses and resolutions.

The book has been published in an excellent get-up in paper-bound.

K. VUKOV

Recent achievements in cryoengineering "Cryoprague 86"

International Institute of Refrigeration, Paris, 1986, 215 pp.

The book contains the proceedings of the A 1/2 meeting held on September 8—12, 1986 in Prague by the International Institute of Refrigeration.

The extracts of 30 presentations given by researchers of 16 countries in the field of cryophysics and cryoengineering are divided into 4 areas.

The first is "Magnetic separation using superconducting magnets". There are 10 presentations about theoretical aspects and a review of magnetic separation systems, high gradient magnetic separator (HGMS), open gradient magnetic separator (OGMS), computation of magnetic forces, calculation of quench parameters, laboratory research for separation of brown coal and kaolin, process testing of a pilot plant machine used for the separation of kaolin.

The second area is „Cryogenic aspects of the NMR imaging and other medical applications." There are 3 presentations about systems for nuclear magnetic resonance

(NMR) imaging, appliances for the treatment of tumours diseases by local undercooling and pecularities of handling cryogenic products.

The third area is "Refrigerators (including miniature and magnetic refrigerators)". There are 3 presentations on magnetic refrigeration, investigation of wave cryogenerator (WCG) and the operation problems of small helium expansion turbines.

The fourth area is "Other new developments and achievements in cryoengineering below 20 K". There are 14 presentations about the investigation and experimental study of cryogenics and large superconductors (CS), thermal and magnetic correlation in apparent strain, special applications of superconducting quantum interference devices (SQUID), thermophysical properties of insulation materials, superinsulation for external pressure load and a pressure gange for the measurement of low pressures.

The book is recommended for researchers, engineers, teachers and students who are interested by progress in the physics of very low temperatures.

M. MÉSZÁROS

ANNOUNCEMENTS

2nd EFFoST EUROPEAN CONFERENCE OF FOOD SCIENCE AND TECHNOLOGY in BRUSSELS on APRIL 9—11, 1991

The Secretariate of the Conference announces that the 2nd EFFoST European Conference of Food Science and Technology has been postponed for one year to 9—11 April 1991 intstead of 1990.

The Scientific Committee is preparing the programme and will send you the definite version as soon as possible.

This news has been given by Prof. Dr. Ing. J. Lenges (C. E. R. I. A., avenue Emile Gryzon 1, B-1070 Brussels).

*

QUALITY AND THE FOOD INDUSTRIES
EUROPEAN SYMPOSIUM
OCTOBER 18TH AND 19TH, 1990

CONSEIL REGIONAL D'ILE DE FRANCE
REGIONAL COUNCIL OF ILE DE FRANCE

33, rue Barbet de Jouy, 75700 Paris

Organizing Committee

Chairman J. M. CLEMENT
Ch. PILET

General Secretary F. H. BOLNOT

Registration fees: full payment at registration 2 000 FF
(all payments must be made in French Fracs only)

Mailing address: Secretariat du C. R. I. T. T.—.I. A. A. I. de F. E. N. V. A., avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex

Telephone: (1) 43.96.25.76
Telefax: (1) 48.93.19.20
Telex: 262 438 CRITTIA

RECENTLY ACCEPTED PAPERS

Peroxidation and heavy metals of dry nuts oil
SATTAR, A., JAN, M., AHMAD, A. & DURRANI, B. K.

The assessment of zearalenone exposition of the Hungarian population in connection with Fusarium infected cereals
KOUDELA, S., SOÓS, K., SOHÁR, J.& BIRÓ, G.

Analytical studies into radiation-induced starch damage in black and white peppers
FARKAS, J., SHARIF, M. M. & BARABÁSSY, S.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 19 No. 2 — 1990

CONTENTS

Index: 26.039

ACTA ALIMENTARIA

An International Journal of Food Science

Editor
J. HOLLÓ

Volume 19 **September 1990** **Number 3**

Akadémiai Kiadó
Budapest

ISSN 0139—3006
CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1990: Volume 19 (4 issues)
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 19

1990

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 19 (3), pp. 221–224 (1990)

THE OCCURRENCE OF MYCOTOXINS IN SOME DRY FRUITS RETAIL MARKETED IN NAINITAL DISTRICT OF INDIA

J. SAXENA[a] and B. S. MEHROTRA[b]

[a] Department of Botany, Kumaun University, Nainital-263002. India
[b] Botany Department, University of Allahabad, Allahabad. India

(Received: 9 November 1988; revision received: 16 November 1989; accepted: 17 November 1989)

Ten samples of marketed dry fruit were screened for various mycotoxins viz., aflatoxins, rubratoxin, ochratoxin A, citrinin, zearalenone and sterigmatocystin. It was found that percentage contamination by mycotoxins was highest in coconut (55.6%) and lowest in date palm (16.7%). Of all mycotoxins, aflatoxins were more common than others and in most of the samples the amount was found to be higher than the permissible limit for human consumption. However, the frequency of occurrence of other mycotoxins was considerably lowed.

Keywords: dry fruits, aflatoxins, ochratoxin A, citrinin, zearalenone, rubratoxin, sterigmatocystin

The influence of naturally occurring mycotoxin residues has had a major impact on the food industries of the world. The ultimate concern is that most of the mycotoxins adversely affect the human health (SPENSLEY, 1963; JOFFE & LISKER, 1969; BHAT et al., 1978). Dry fruits which are among expensive commodities due to their high food value and production in restricted regions, are mostly used raw in confectioneries. If contaminated nuts, raw or processed, are consumed, it may cause health disorder in consumers, nonetheless, no systematic, in-depth survey has been carried out in India emphasizing the need of their screening for mycotoxins in this subcontinent. Although there have been some studies in India and abroad confined to the screening of groundnut and coconut for aflatoxins, the other dry fruits which are also commonly consumed, and mycotoxins other than aflatoxins which are equally hazardous for human health have not been given due consideration. Hence the present study was planned to screen the marketed dry fruits for various mycotoxins.

1. Materials and methods

The samples of ten dry fruits viz., walnut (*Juglans regia* Linn.), coconut (*Coccus nucifera* Linn.), cashew nut (*Anacardium occidentale* Linn.), groundnut (*Arachis hypogea* Linn.), raisin (*Vitis vinifera* Linn.), almond (*Prunus amygdalus* Batsch.), cuddapah almond (*Buchnania lanzan* Spreng), date palm (*Phoenix dactylifera* Linn.), pistachio nut (*Pistacia vera* Linn.) and gorgannut (*Euryale ferox* Salisb.) were obtained randomly in sterilized polythene bags from all major local retail outlets at Nainital district during 1983–85. The frequency of sampling during the three year period was 6–9, shown as number of samples in Table 1. Each sampling time was different. The samples which were not apparently infested were specifically selected as the commodities showing visible mould growth are generally not purchased by consumers. The sample size was 250 g which was thoroughly mixed before screening. Twenty five g of sample was analysed by the methods of PONS and co-workers (1966), SÁNDOR (1982) and PATTERSON and ROBERTS (1979) for the natural contamination of aflatoxin, rubratoxin and remaining mycotoxins, respectively. The number of replicates for each sample was 3. The quantitative estimation was done by the method employed by DETROY and co-workers (1971). Due to the unavailability of standards of ochratoxin A and zearalenone, their quantitative estimation could not be executed but the chemical confirmation of all the toxins was carried out.

2. Results

The statistically analysed data are given in Table 1. The type and quantity of the mycotoxin varied with different dry fruits. The percentage of contaminated samples was the highest in coconut (55.6 %) followed by groundnut (50.0 %) and the lowest in date palm (16.7 %).

Of all the mycotoxins for which dry fruits were tested, aflatoxin was more common than others. It was found in 28 out of 74 samples of different dry fruits. The ratio of aflatoxin B_1 and B_2 was reported to be too small to compare to usual results reported. Among other mycotoxins citrinin was present in 7 samples, ochratoxin A in 6, sterigmatocystin in 4, and rubratoxin and zearalenone each in 2 out of 74 samples screened. Often more than one mycotoxin were present in a sample.

3. Conclusions

Among all the dry fruits analysed coconut was the most contaminated one. ARSECULERATNE and co-workers (1969) have suggested that in coconut the fatty acids, carbohydrates or toxin stabilising factors might favour the

Table 1

Natural occurrence of mycotoxins in different dry fruits
(Total number of samples: 74)

Dry fruits	Number of samples contaminated	Specific mycotoxin detected	Number of samples contaminated with specific mycotoxin	Quantity of mycotoxin (μg per kg)
Coconut	9	Afl.	5	28±2.25 to 260±13.01 B_1, 15±0.71 to 109±4.58 B_2, 12±5.03 to 75±5.23 G_1, 20±1.52, 68±2.61 G_2
		Cit.	4	5±1.52 to 60±4.60 cit.
Raisin	8	Afl.	3	15±1.42 to 180±5.50 B_1, 16±1.75, 150±8.50 B_2, 15±2.29, 80±2.46 G_1, 15±1.05 G_2
		Ochr.	1	+ ochr.
Cuddapah almond	6	Afl.	2	25±2.25, 71±5.14 B_1, 17±1.47 B_2, 25±1.80 G_1
		Zear.	1	+ zear.
		Rub.	1	310±15.50 rub.
Pistachio nut	6	Afl.	2	15±1.85, 94±3.9 B_1, 75±3.20 B_2, 12±2.06 G_1, 8±1.95 G_2
		Stg.	2	70±5.0, 100±5.51 stg.
Groundnut	6	Afl.	3	20±1.90 to 200±19.51 B_1, 10±1.05, 135±9.01 B_2, 12±2.0, 96±3.20 G_1, 10±2.52 G_2
		Ochr.	2	+ ochr.
Almond	8	Afl.	3	18±2.51 to 194±6.55 B_1, 20±1.75, 125±7.0 B_2, 19±3.0, 30±3.95 G_1
		Zear.	1	+ zear.
		Stg.	2	96±4.50, 130±7.50 stg.
Cashew nut	7	Afl.	3	20±2.96 to 190±9.50 B_1, 15±2.36 to 161±10.5 B_2, 18±3.51, 72±4.51 G_1, 20±2.51 G_2
		Ochr.	2	+ ochr.
		Cit.	1	40±3.51 cit.
Walnut	9	Afl.	3	15±3.0 to 110±9.01 B_1, 12±1.25 to 85±4.51 B_2, 10±2.05, 75±6.0 G_1, 65±5.50 G_2
		Rub.	1	210±9.50 rub.
Datepalm	6	Afl.	1	19±2.51 B_1, 15±0.90 B_2, 14±1.95 G_1
		Ochr.	1	+ ochr.
Gorgannut	9	Afl.	3	20±2.51 to 150±8.50 B_1, 15±0.99, 75±6.50 B_2, 16±3.05, 76±7.50 G_1, 10±1.75 G_2
		Cit.	2	4±0.85, 20±5.50 cit.

Afl.: Aflatoxin, E_1: Aflatoxin E_1, E_2: Aflatoxin E_2, G_1: Aflatoxin G_1: G_2; Aflatoxin G_2, Cit.: Citrinin, Ochr.: Ochratoxin A, Zear.: Zearalenone, Stg.: Sterigmatocystin, Rub.: Rubratoxin, +: present

Figures denote mean values of 3 replicates ± standard deviation

production of toxin. Of all the mycotoxins the aflatoxins were the most frequent. The maximum permissible level for aflatoxin in groundnut destined for human consumption have been set at 15 μg per kg in the USA (STOLOFF, 1977) which has been recently fixed at 20 μg per kg by World Health Organisation. It is alarming that in most of the contaminated samples the quantity was higher than the above permitted limit.

It is explicit from the results that mycotoxins other than aflatoxins were present only in a few samples. Thus the often quoted production of these mycotoxins in large amounts in laboratory cultures does not translate into a high frequency of occurrence under natural conditions especially in the marketed dry fruits. The presence of all these mycotoxins in dry fruits in substantial amount is noteworthy as they are often consumed raw. Thus appropriate measures should be taken to overcome this problem.

*

Authors are thankful to Drs. B. A. ROBERTS, Central Veterinary Laboratory, Surrey, U. K.; P. KROUGH, Royal Veterinary and Agricultural University, Denmark and G. S. SÁNDOR, Central Veterinary Institute, Budapest for the generous supply of mycotoxin standards.

Literature

ARSECULERATNE, S. N., DE SILVA, L. M., WIJESUNDERU, S. & BANDHUNATHA, C. H. S. R. (1969): Coconut as a medium for the experimental production of aflatoxin. *Appl. Microbiol., 18*, 88–94.

BHAT, V. R., NAGARAJAN, V. & TULPULE, P. G. (1978): *Health hazards of mycotoxins in India.* Nat. Inst. Nutr., Hyderabad (ICMR), p. 58.

DETROY, R. W., LILLEHOJ, E. D. & CIEGLER, A. (1971): Aflatoxin and related compounds. -in: CIEGLER, A , KADIS, S. & AJL, S. J. (Eds) *Microbial toxins.* Academic Press, New York, p. 178.

JOFFE, A. Z. & LISKER, N. (1969): Effect of light, temperature and pH value on aflatoxin production in vitro. *Appl. Microbiol., 18*, 517–518.

PATTERSON, D. S. P. & ROBERTS, B. A. (1979): Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxins, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin. *J. Assoc, off. anal. Chem., 49*, 554–562.

PONS, W. A. JR., CUCULLU, A. F., LEE, L. S., ROBERTSON, J. A., FRANZ, A. O. & GOLDBLATT, A. (1966): Determination of aflatoxins in agricultural products: Use of aqueous acetone for extraction. *J. Assoc off anal. Chem., 49*, 554–462.

SÁNDOR, G. S. (1982): Rubratoxin B: Thin layer chromatographic determination in corn, effect of incubation time, effect of wheat on occurrence. *Proc. Symp. Mycotoxins*, pp. 423–429.

SPENSLEY, P. C. (1963): Aflatoxins, the active principle in Turkey 'X' disease. *Endeavor, 22*, 75–79.

STOLOFF, L. (1977): Aflatoxins an overview. -in: RODRICS, R. V., HESSELTINE, C. W. & MEHLMAN, M. A.: *Mycotoxins in human and animal health.* Pathotox Publishers, p. 7.

Acta Alimentaria, Vol. 19 (3), pp. 225–228 (1990)

PEROXIDATION AND HEAVY METALS OF DRY NUTS OILS

A. SATTAR, M. JAN, A. AHMAD and S. K. DURRANI

Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan

(Received: 13 February 1989; revision received: 24 November 1989; accepted: 29 November 1989)

Influence of different packages on the oxidation rates of almond, peanut, pinenut and walnut oils during exposure to fluorescent light, was studied. Concentrations of heavy metals such as zinc, copper, lead and cadmium were determined. It was observed that peroxidation (POV) of these oils was greatly increased during light exposure ($P < 0.05$) and the overall order was walnut > pinenut > peanut > almond. Amber glass afforded maximum protection against the photooxidation. There were marked variations in the content of heavy metals (CV 35.6–66.1).

Keywords: dry nut oils, metals, peroxidation, photooxidation, fluorescent light, packages

Dry nuts are considered a major source of income and foreign exchange in Pakistan and manycountries of the world. During solar drying, they undergo colour, flavour and chemical changes especially under tropical conditions such as exist in Pakistan. Light induced reactions have been shown to affect the stability of fatty foods (SATTAR & DEMAN, 1975). Effect of heat and different packages on stability of ground nut oils has been reported (MAHADEVAIAH et al , 1982; NASIRULLAH et al., 1982; RIKHTER, 1983). Although peroxidation of irradiated dry nuts during storage at varying temperatures has been studied (JAN et al., 1988; SATTAR et al., 1989c), peroxidation and heavy metals in dry nut oils has been less tested, which is the object of these studies.

1. Materials and methods

Dry nuts such as almond, peanut, pinenut and walnut were obtained from the market at Peshawar. The oils were Soxhlet extracted using petroleum ether (b.p. 40–60 °C). The solvent was initially removed with the help of rotary evaporator at 40 °C and then in an air oven at 40–50 °C. Oil samples were placed at ambient temperature (25–35 °C) and exposed to fluorescent light intensity of 43 lux, which was measured by means of a General Electric type–214 light meter. Control samples were kept for corresponding time-temperature conditions in the dark.

Peroxidation of oils was measured by the peroxide value according to the AOCS (1972) method (cd-8–35). After wet digestion of the samples (SATTAR

& CHAUDRY, 1978), simultaneous determination of heavy metals such as Cd, Cu, Pb and Zn was carried out by the potentiometric stripping technique (DANIELSSON et al., 1983) using Tecator Striptec system–1069–001 comprising glass carbon electrode, saturated calomel electrode (SCE) and platinum wire as counter electrode. The stripping curves were measured at a potential of −1.1V vs SCE 180 s plotting time for Cd, Cu and Pb, while −1.3V vs SCE and 180 s for Zn. Statistical analysis of the data on peroxidation was done by analysis of variance. An estimate of the variation in the relative rate of peroxidation and heavy metal contents of oils was made by determining the coefficient of variation (CV), which is a ratio in percentage of standard deviation to the mean (LITTLE & HILLS, 1972).

2. Results and discussion

Influence of different packages and fluorescent light on peroxide values of different dry nut oils is shown in Table 1. It was observed that peroxidation increased with advance of storage, and exposure to light markedly accelerated peroxidation. Development of peroxide value was greatly higher in exposed samples. Obviously amber glass protected the oil against photo-oxidation better than clear glass and the extent of protection was almost identical to unexposed control. The rate of peroxidation in the oil occurred in the following order: walnut > pinenut > peanut > almond. Statistical treatment of data revealed significant effect of light and packages ($P < 0.05$). In order to make an estimate of dispersion of peroxide values in relation to packaging materials, the coefficient of variation (CV) was measured. This revealed striking differences in the peroxide values. Determination of the CV is especially

Table 1

Effect of packages on peroxide values (Meq kg^{-1}) *of dry nut oils exposed to 43 lx of fluorescent light at ambient temperatures*

Packages	Stored product							
	Almond		Peanut		Walnut		Pinenut	
	Storage period (week)							
	3	6	3	6	3	6	3	6
Exposed control	11.0	37.5	14.5	38.5	30.2	55.1	23.0	50.7
Clear glass	9.5	20.8	11.8	18.9	27.5	40.0	20.0	43.5
Amber glass	4.0	5.5	4.9	6.3	8.0	10.1	7.5	9.5
Unexposed control	3.9	4.8	3.9	5.3	6.0	8.2	5.5	8.6
Mean	7.1	17.2	8.8	17.3	17.9	28.4	14.0	28.1
CV	52.0	90.1	59.1	89.6	70.8	81.2	62.8	79.0

$LSD_{0.05\%}$: treatments (1.16); storage time (0.92)
Initial POV: almond oil 2.8, peanut oil 2.7, pinenut oil 2.6 and walnut oil 3.4 Meq per kg
Ambient temperature: 25–35 °C

appropriate under conditions where there are extreme values or when it is desired to express variation as a percentage of the average around which the deviations are taken.

Influence of different coloured packages on oxidation of dry nut oils has been less studied. However, recently SATTAR and co-workers (1989b) observed similar pattern at ambient temperatures and 21.5 lux of light intensity. Previously amber glass was found to be better in protecting the quality of liquid milk and orange drinks than clear glass bottles and Tetrapak packages (SATTAR et al., 1983; SATTAR et al., 1989a). It is primarily because clear glass transmits the entire incident light between 300–1100 nm, whereas the amber glass transmitted only 14% mainly between 500–1100 nm (SATTAR et al., 1989b). It was therefore inferred that better protection property of amber glass is due primarily to its ability of complete absorption of the damaging wavelengths of the light spectrum (200–500 nm), and substantial reduction of the incident light. The amber glass also blocked the near IR wavelengths of the spectrum as well. An association between higher peroxidation and shorter wavelengths of light has indeed been reported earlier (SATTAR et al., 1976). In addition to the contribution of fatty acids, the differences in peroxidation of these oils may well be due to natural variation in the content of antioxidants and pigments.

In view of the importance of heavy metals in health and nutrition as well as association of certain heavy metals with the catalysis of oxidation in oils and fats, these dry nut oils were assayed for zinc, copper, lead and cadmium and the results are presented in Table 2.

The data indicated that these oils generally contained appreciable amounts of essential elements such as zinc (8.78–22.90 μg per g) and copper (2.60–7.74 μg per g) and had relatively lower cadmium (0.09–0.24 μg per g) than lead (0.18–1.01 μg per g) contents. Overall results showed that almond oil had larger concentration of these heavy metals than the other oils. Com-

Table 2

Concentration of heavy metals in dry nut oils

Dry nut oil	Heavy metals (μg per g)							
	Zn		Cu		Pb		Cd	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Almond	22.90	1.78	6.19	0.66	1.01	0.05	0.24	0.003
Peanut	19.77	2.65	2.60	0.07	0.49	0.04	0.09	0.010
Pinenut	8.78	0.83	3.93	0.61	0.43	0.02	0.11	0.004
Walnut	22.22	0.26	7.74	0.84	0.18	0.03	0.11	0.001
CV	35.6		44.8		66.1		50.2	

Values are averages of three determinations

parison of the oxidation rates of these oils with their heavy metal concentration indicated little or no association between them. Determination of CV revealed wide differences in the concentration of heavy metal content of spices, dry fruits and plant nuts (SATTAR et al., 1989d). Differences in the concentration of these heavy metals in dry nut oils were considered to be merely a reflection of the microelement content of soils on which these nut plants had been grown, and to a lesser extent of their genetic potential for uptake of these metals. There appears to be good reason to assume that light induced peroxidation of nut oils depends generally on the natural unknown inhibitors as shown in the case of other oils and fats (SATTAR et al., 1976; LUNDBERG, 1962). As a result of these studies it was observed that dry nut oils greatly vary in their heavy metal concentrations and that amber glass due to its ability of absorbing the harmful wavelengths of the light spectrum, is a suitable package for their long time storage.

Literature

AOCS (1972): Official and tentative methods of the American Oil Chemists Society. Champaign, I. USA.

DANIELSSON, L. G., JAGNER, D., JOSEFSON, M. & WESTERLUND, S. (1983): Computerized potentiometric stripping analysis for the determination of cadmium, lead, copper and zinc in biological materials. *Analytica chim. Acta, 127*, 147–156.

JAN, M., LANGERAK, D. IS., WOLTERS, T. H. D., FARKAS, J., KAMP, H. J. V. D. & MUUSE, B. G. (1988): The effect of packaging and storage conditions on the keeping quality of walnuts treated with disinfestation doses of gamma rays. *Acta Alimentaria, 17*, 13–31.

LITTLE, J. M. & HILLS, F. J. (1972): Statistical methods in agriculture research. Agric. Extension. Univ. California, Davis. USA.

LUNDBERG, W. O. (1962): Lipids and their oxidation. (H. W. SCHULTZ, E. A. DAY and R. O. SINNHUBER, Eds.) AVI, West Port, CT, pp. 31–50.

MAHADEVAIAH, B., BALDEVRAJ, V. R. & ANANDESWAMY, B. (1982): Studies of raw ground nut oil in flexible pouches. *J. Indian Fd Packer, 36*, 78–80.

NASIRULLAH, A., KRISHNAMURTHY, M. N., NAGARJA, K. V. & KAPUR, O. P. (1982): Storage study on ground nut oil. *J. Oil Technol. Ass. India, 14*, 55–56.

RIKHTER, A. A. (1983): Oxidation of almond and walnut oil. *J. appl. Biochem. Microbiol., 16*, 446–450.

SATTAR, A. & CHAUDRY, M. A. (1978): Trace element contents of food and their interrelationships with protein values in milled fractions of wheat and triticale. *Pakist. J. Biochem., 11*, 48–54.

SATTAR, A. & DEMAN, J. M. (1975): Photo-oxidation of milk and milk productions. *CRC Crit. Rev. Fd Sci. Nutr., 7*, 13–37.

SATTAR, A., DEMAND, J. M. & ALEXANDER, J. C. (1976): Effect of wavelength on light induced quality deterioration of edible oils and fats. *Can. Inst. Fd Sci. Technol. J., 9*, 108–113.

SATTAR, A., DURRANI, M. J., KHAN, R. N. & HUSSAIN, E. (1989a): Effect of packaging materials and fluorescent light on HTST-pasteurized orange drink. *Z. Lebensmittelunters. u.-Forsch., 188*, 430–433.

SATTAR, A., JAN, M.. AHMAD, A., HUSSAIN, A. & KHAN, I. (1989b): Light induced oxidation of nut oils. *Nahrung, 33*, 213–215.

SATTAR, A., JAN, M., AHMAD, A., WAHID, M. & KHAN, I. (1989c): Irradiation disinfestation and biochemical quality of dry nuts. *Acta Alimentaria, 18*, 45–52.

SATTAR, A., TAVANGER, R. & AHMAD, S. (1983): Effect of packaging materials on photocatalysed changes in buffalo milk. *Z. Lebensmittelunters u. -Forsch., 117*, 121–123.

SATTAR, A., WAHID, M. & DURRANI, S. K. (1989d): Concentration of selected heavy metals in spices, dry fruits and plant nuts. *Pl. Fd Human Nutr.* (In press.)

Acta Alimentaria, Vol. 19 (3), pp. 229—235 (1990)

THE ASSESSMENT OF ZEARALENONE EXPOSITION OF THE HUNGARIAN POPULATION IN CONNECTION WITH FUSARIUM INFECTED CEREALS

S. KOUDELA, K. SOÓS, J. SOHÁR and G. BÍRÓ

National Institute of Food Hygiene and Nutrition,
H-1097 Budapest, Gyáli út 3/a. Hungary

(Received: 23 February 1989; revision received: 15 June 1989; accepted: 2 October 1989)

A nation-wide survey was carried out to establish the zearalenone contamination of edible wheat and of its milling products in co-operation with the National Institute of Food Hygiene and Nutrition and the County Hygienic and Epidemiological Stations. On the basis of the data obtained, the possible zearalenone intake of the population was estimated.

In the majority (98.3%) of the about 300 samples, taken in an average year according to cultivation conditions, zearalenone levels were below the detection limit (< 40 μg kg^{-1}) in five samples (1.7%) zearalenone levels were between 40 and 120 μg kg^{-1}.

According to the average zearalenone levels of wheat and its milling products, it is presumed that the zearalenone intake of the population due to wheat products does not exceed a daily amount of 0.2 μg per kg body mass and this value is 100-fold lower than the maximum no effect level established for the most sensitive animal species in respect of reproduction.

Keywords: zearalenone exposition, Fusarium infected wheat

The risk of cereal being infected with Fusarium exists all over the world. Fusarium produced mycotoxins are relatively frequently detected in feed samples, analysed because of animal diseases (SÁNDOR, 1984, VÁNYI, 1987). The amount of zearalenone may reach health hazard levels under unfavourable conditions of cultivation (weather) and storage in feed wheat and corn (LÁSZTITY & WÖLLER, 1975).

Zearalenone's estrogenic effect and the concomitant infertility and death have caused great losses in livestock breeding. The toxin may get with cereal products into the human organism, thus its role in barrennes (in spontaneous abortion) cannot be excluded.

The occurrence of zearalenone has been reviewed in many publications and data have been surveyed in detail (KUIPER–GOODMAN et al., 1987). (Most of the data relate to corn, more sensitive to Fusarium infection). Wheat samples from Virginia (USA) examined between 1976 and 1980 did not contain zearalenone while 45% of the samples studied in 1975 was positive (SHOTWELL & HESSELTINE, 1983).

According to another report, in 17 wheat samples out of 112, examined in the USA in 1975, zearalenone occurred at ppm level and in 2 samples above 5 mg kg^{-1} (BENNETT & SHOTWELL, 1979). In Czechoslovakia, 46% of the 28 samples investigated was positive and the maximum level found was 261 μg kg^{-1} (BARTOŠ & MATYÁŠ, 1981). In Korea, in two out of ten polished wheat samples 8–40 μg kg^{-1} zearalenone levels were detected (LEE et al., 1985). In three Japanese wheat flour samples out of 27, 1–6 μg kg^{-1} zearalenone was found (TANAKA et al., 1985). The higher values detected in samples from the USA and Czechoslovakia are most likely due to mouldy produce or to a rainy period during the growth period. Considering the health hazards involved in the consumption of contaminated wheat and the toxic properties of zearalenone, it was decided as part of the control programme of Hungarian foods, to collect data on the zearalenone infection of wheat and wheat products, playing an important role in food consumption in Hungary.

1. Materials and methods

1.1. Materials

1.1.1. Samples from milling plants. Edible wheat and its milling products taken from milling plants: 58 grain samples; 202 samples of flour and semolina; 42 bran samples, making altogether 302 samples. The samples were representative of almost every county (18) and the plants operating in these areas, respectively. The average number of samples investigated in the laboratories of the National Institute of Food Hygiene and Nutrition and of the Hygienic and Epidemiological Stations, was 16 (range 3–43). Preference was given to flour samples because being ground, they were easy to homogenise and were ready for analysis.

1.1.2. Samples from wheat fields. Nine samples of mature wheat ready for harvesting, taken from an area considered infected, in county Tolna, from 3 different villages, representing one plot of land each.

From the same infected area, in 2 ear samples, the grains visibly infected were separated from the healthy ones and both groups as well as the chaff got analysed separately.

One of the infected wheat samples was neither dried nor immediately processed and thus in closed packing it got heated during one week's stay.

1.2. Sampling

Samples were taken according to the specifications of the related standards (HUNGARIAN STANDARD, 1980). Laboratory samples of 1 kg were separated from lots representing 1 tons of wheat awaiting milling or already milled (flour), or about 0.25 ha of wheat fields.

1.3. Determination of zearalenone

Zearalenone was determined by thin-layer chromatography, a method easy to carry out in all the participating laboratories (MIROCHA et al., 1974). Fifty g from the homogenized sample was shaken on a jolting machine for 1 hour with 250 cm^3 dichloromethane and 15 cm^3 water. The extract was filtered and washed twice with 25 cm^3 dichloromethane each. The filtrate and the washings were combined and then evaporated till dry. The residue was dissolved in 20 cm^3 chloroform and the zearalenone was extracted first with 10, then with 5 cm^3 1 *N* NaOH (the alkaline solution was washed with 15 cm^3 chloroform and this was discarded). The pH of the aqueous phase was set 9.5 and the phase was extracted three-times with chloroform of 15 cm^3. The extract was dried over anhydrous Na_2SO_4, then evaporated till dry.

The residue was dissolved in an appropriate amount of benzene, generally 0.5 cm^3 and was applied to a silica-gel layer (MN 25 G HR), it was developed in the mixture of chloroform and ethanol (96.5 + 3.5). The spots were evaluated on the basis of their fluorescence in UV light of 254 nm.

To confirm the results, detection in methoxybenzene-diazonium-fluoroborate (SARUDI, 1974) or in other developing systems was carried out, too.

The method was checked in all the participating laboratories. Detection limits were between 20 to 40 $\mu g\ kg^{-1}$, recoveries were between 60–80 %.

2. Results

In the majority of the 302 samples obtained from the mills, zearalenone levels were below the 40 $\mu g\ kg^{-1}$ detection limit. Only in 5 samples (1.7 %) were found zearalenone levels exceeding this limit (Table 1).

Out of the 9 samples taken from an infected area, in 5 samples (56%) zearalenone levels did not exceed the detection limit (40 $\mu g\ kg^{-1}$), in the remaining 4 samples (44%) the result was positive (56, 64, 300 and 800 $\mu g\ kg^{-1}$ zearalenone).

In the ear of wheat (Table 2), in grains visibly infected, zearalenone amounted to several thousands of $\mu g\ kg^{-1}$. In grains, visibly judged healthy, zearalenone was around the detection limit or slightly above.

In the sample of heated grain, zearalenone content was 90 mg kg^{-1}.

Table 1

Zearalenone in wheat and its milling products

Product	Number of samples		Per cent of positive samples	Zearalenone ($\mu g\ kg^{-1}$)	
	total	positive		$\bar{x}$	$\pm s$
Grain	58	0	0		—
Flour	202	3	1.48	50	5.9
				60	11.8
				120	23.6
Bran	48	2	4.17	75	17.7
				100	29.7
Total	302	5	1.65		—

Table 2

Distribution of zearalenone in infected ear of wheat ($\mu g\ kg^{-1}$)

In healthy grain		In contaminated grain[a]		In chaff	
$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
33	9.8	3200	708	180	35.4
67	14.7	5900	1062	100	23.6

[a] Proportion in samples 8.5 and 2.7% respectively

3. Discussion

3.1. Samples from milling plants

The most important conclusions, that is the assessment of health hazard of the population, were drawn from the results obtained in wheat samples and in their milling products. Zearalenone content in the majority of the samples did not reach the 40 $\mu g\ kg^{-1}$ detection limit. Thus, because of the low number of positive samples and their low zearalenone content, the average level of zearalenone in edible wheat and in its milling products is also below the detection limit.

These results relate to an average year from the point of view of both weather conditions and the so called inner Fusarium infection of the produce.

The results reflect mainly the state of wheat flour because of the few bran samples. However, from the aspect of food hygiene it has to be taken into account that in spite of the higher proportion of positive bran samples, the consumption of bran is very low as compared to flour.

Table 3

Data on zearalenone intake of the population

Country	$\mu g\ kg^{-1}$ body mass per day	$\mu g\ kg^{-1}$ diet	Literature
Canada	0.05–0.1	10–20	KUIPER–GOODMAN et al., 1987
Hungary	<0.2	<40	Data in this paper

Table 4

Maximum no effect levels of zearalenone in respect of reproduction

Animal species	mg kg^{-1} body mass	mg kg^{-1} feed	Literature
Rat	0.85	17	RUZSÁS et al., 1979
Pig	0.37	25	CHANG et al., 1979
Sow	0.06	3	YOUNG and KING, 1984

The fact, that in our investigations both zearalenone levels and occurrences were low, was possibly affected by wheat not being the most sensitive substrate (HALÁSZ et al., 1986).

Considering the general zearalenone contamination of flour (below 40 $\mu g\ kg^{-1}$), calculating with 250 g daily flour consumption and an average body mass of 50 kg, the daily zearalenone consumption of the population does not exceed 0.2 $\mu g\ kg^{-1}$ body mass. Our data are compared with relevant Canadian data (KUIPER–GOODMAN et al., 1987) in Table 3.

The health hazard of the daily intake of 0.2 $\mu g\ kg^{-1}$ body mass zearalenone can be evaluated by comparing these data with those found in the literature (Table 4).

Thus, the following conclusions can be drawn: the presumed zearalenone intake of the population is 100-fold lower than the maximum no effect level established for the most sensitive animal species in respect of reproduction.

3.2. Samples from infected wheat plots

Among the wheat samples taken from the plots considered infected, a part of the samples contained several hundreds of $\mu g\ kg^{-1}$ zearalenone within the ear, while low in number but containing toxin at high level, may contaminate a bigger batch. At the same time, the grains healthy in appearance, contained also a measurable amount of zearalenone. According to these investigations, at harvest time contamination is not evenly distributed in a plot. Since every batch of the harvested grain cannot be tested for zearalenone infection,

it seems very important to avoid the storage of grains, however lightly contaminated.

This fact is of special importance as examinations of inocula (BADAWEY, 1986) indicate that relatively low mould counts may yield high toxin production in favourable conditions.

The high toxin content of the heated sample points to the danger of rapid spreading of contamination. In this case, toxin production did not need the arrival of cold weather—considered generally as a precondition.

To keep infection and contamination at low levels, it is necessary to carry out the preliminary fungicide treatment in time, to meet the purchase requirements and to perform cleaning operations before storage in mills.

For the sake of taking precautions against years of higher contamination due to more rainy weather, the separate harvesting of crops of different qualities has to be organised with great care and it is necessary to increase storage capacity in order to be able to provide for separate storage, as specified in the standard.

*

The authors wish to express their thanks for sampling and participating in the collaborative study to the members of the laboratories and for the methodological work to Ms É. BARANYI.

Literature

BADAWEY, A. (1986): Influence of mycotoxin production affecting factors on the F-2, T-2 and ochratoxin contamination of grains. Ph. D. Dissertation. Central Food Research Institute, Budapest.

BARTOŠ, J. & MATYÁŠ, Z. (1981): Výskyt zearalenonu v tuzemských zrninách. (The occurence of zearalenone in grains produced in Czechoslovakia.) *Vet. Med., Praha, 26*, 505–512.

BENNETT, G. A. & SHOTWELL, O. L. (1979): Zearalenone in cereal grains. *J. Amer. Oil Chem. Soc., 56*, 812–819.

CHANG, K., KURTZ, J. E. & MIROCHA, C. J. (1979): Effects of the mycotoxin zearalenone on swine reproduction. *Amer. J. Vet. Res., 40*, 1260–1267.

HALÁSZ, A., BADAWEY, A., SAWINSKY, J. & KOZMA, E. (1986): F-2 toxintermelő penésztörzsek zearalenon képzése különböző szubsztrátumokon. (Zearalenon formation of F-2 toxin producing mould strains in different substrata.) *Élelm. Ipar, 40*, 47–49.

HUNGARIAN STANDARD (1980): Mezőgazdasági termények és növényi termékek mintavétele. (Sampling of agricultural products and materials of plant origin.) MSZ 14475/14–80.

KUIPER-GOODMAN, T., SCOTT, P. M. & WATANABE, H. (1987): Risk assessment of the mycotoxin zearalenone. *Regul. Toxicol. Pharmacol., 7*, 253–306.

LÁSZTITY, R. & WÖLLER, L. (1975): Toxinbildung bei Fusarium-Arten und Vorkommen der Toxine in Landwirtschaftliche Produkten. *Nahrung, 19*, 537–546.

LEE, U. S., JANG, H. S., TANAKA, T., HASEGAWA, A., OH, Y.-J. & UENO, Y. (1985): The coexistence of the Fusarium mycotoxins nivalenol, deoxynivalenol and zearalenone in Korean cereals harvested in 1983. *Fd Addit. Contam., 2*, 185–192.

MIROCHA, CH. J., SCHAUERHAMMER, B. & PATHRE, S. V. (1974): Isolation, detection and quantitation of zearalenone in maize and barley. *J. Ass. off. anal. Chem., 57*, 1104–1108.

RUZSÁS, C., BÍRÓ-GOSZTONYI, M., WÖLLER, L. & MESS, B. (1979): Effect of the fungal toxin (zearalenone) on the reproductive system and fertility of male and female rats. *Acta Biol. Acad. Sci. Hung., 30*, 335–345.

SARUDI, I. (1974): Methode zum Nachweis des Zearalenone (F-2) toxins. *Z. Lebensmittel-unters. u. -Forsch., 154*, 65–69.

SÁNDOR, G. (1984): Occurrence of mycotoxins in feeds, animal organs and secretions. *Acta Vet. Acad. Sci. Hung., 32*, 57–69.

SHOTWELL, O. L. & HESSELTINE, C. W. (1983): Five-year study of mycotoxins in Virginia wheat and dent corn. *J. Ass. off. anal. Chem., 66*, 1466–1469.

TANAKA, T. HASEGAWA, A., MATSUKI, Y. & UENO, Y. (1985): A survey of the occurrence of nivalenol, deoxynivalenol and zearalenone in foodstuffs and healthy foods in Japan. *Fd Addit. Contam., 2*, 259–265.

VÁNYI, A. (1987): A Magyarországon előforduló fontosabb mikotoxinok és mikotoxikózisok. Tanulmány a „Környezet és Egészség Bizottság" ülésére. (The more important mycotoxins and mycotoxicoses occuring in Hungary. A study for the Comittee "Environment and Health".) Hungarian Academy of Science, Budapest.

YOUNG, L. G. & KING, G. J. (1984): Zearalenone and swine reproduction. *J. Amer. Vet. Med. Assoc., 185*, 334–335.

Acta Alimentaria, Vol. 19 (3), pp. 237–260 (1990)

INVESTIGATION OF THE RELATIONSHIP BETWEEN WHEAT LIPIDS AND BAKING PROPERTIES

E. M. KÁRPÁTI[a], F. BÉKÉS[a, c], R. LÁSZTITY[a], F. ÖRSI[a], I. SMIED[a] and Á. MOSONYI[b]

[a] Department of Biochemistry and Food Technology, Technical University of Budapest, H-1521 Budapest, Pf. 91. Hungary
[b] Research Institute for Milling Industry H-1024 Budapest, Kisrókus u. 16/b. Hungary

(Received: 18 April 1989; accepted: 25 September 1989)

Baking performance, whole meal and flour lipid content and composition were determined on samples of eight Hungarian "Martonvásár" wheat cultivars grown at seven different locations in Hungary. Free and bound lipids (extracted with n-hexane and water saturated n-butanol, respectively) were isolated then separated into nonpolar lipid, glycolipid and phospholipid fractions using silicic acid column chromatography. Lipid compositions of samples were determined using thin-layer chromatography. Functional properties were characterized by valorigraph test, Hagberg falling number, sedimentation tests (Zeleny and SDS) and baking test.

Different statistical methods (simple and multiple stepwise regression, analysis of variance, cluster analysis) were carried out to investigate the effects of genetical and enviromental factors on the variations of analytical and functional parameters investigated. Relations of lipid data and loaf volume were characterized, as well, to find relationships suitable to predict loaf volume from lipid data as a rapid screening test in breeding selection.

Detailed chemical composition data of wheat and flour lipids of Hungarian wheats presented in this paper show that their variations are caused mainly by environmental factors.

Highly significant correlations were found between loaf volume potential and different lipid parameters. The strongest relationships were found with the free nonpolar/polar lipid and with the nonpolar/glycolipid ratios ($r = 0.940$ and 0.823, respectively; both are significant at $P = 0.05$).

Multiple linear regression equations — applying protein content, free glycolipid content and free nonpolar/polar ratio of both flour or whole meal samples — were found to be suitable to predict loaf volume not more than 10% error.

Keywords: wheat lipids, baking properties, correlations between lipid composition and baking value of wheats, prediction of baking value of wheats

Wheat flour lipids although minor constituents of flour (generally around 2%), play an important role in determining functional properties. Extractability of lipids with petroleum ether or n-hexane changes drastically during dough-making, lipids are extractable in water-saturated n-butanol only (CHIU & POMERANZ, 1966). This indicates that lipids are involved in the gluten structure. Special subunits of gluten proteins have been found capable of binding flour lipids (BÉKÉS et al., 1983, a, b, LÁSZTITY et al., 1988).

[c] Present address: CSIRO Division of Plant Industries Wheat Research Unit, North Ryde, P.O. Box 7., N.S.W. Australia

Protein-lipid interaction caused by wetting and by mechanical input during dough mixing are the key factors to understanding the effect of wheat lipids on baking properties: this complex structure acts as lubricant in the dough, on the other hand, it is responsible for gas-retaining capability of dough, which is directly related to loaf volume (POMERANZ, 1980).

Recently strong linear relationships were found between loaf volume and some lipid analytical data of wheat or of flour in North American cultivars. Loaf volume showed good correlation to polar lipid content as well as with nonpolar/polar ratio of n-hexane extracted lipids, so the baking properties were predictable from lipid-data (CHUNG et al., 1982; BÉKÉS et al. 1983c; 1986). Because of the fact that the method introduced is cheap, relatively simple and above all requires very small amount of sample, it can be used as a screening method in the early stage of wheat breeding programs (BUSHUK et al., 1985a, b).

The procedure was adapted in several countries using their own varieties and their own baking tests for calibration (BÉKÉS & MACRITCHIE, 1988a, b; BELL et al., 1987.; LARSEN et al., 1985; 1986; MORRISON, 1987; PANOZZO et al., 1987; 1988). These investigations showed that the above mentioned relationships can be found with Australian, English, New-Zealand wheats, as well, however they are not as strong as in the case of North American varieties.

Applications of these methods to Hungarian wheat varieties, namely "Martonvásár" cultivars, are presented here. This is the most detailed investigation of lipid content and composition of Hungarian wheats since the pioneer work of VUK (1929). Other aspects of these investigations were published elsewhere (LÁSZTITY et al., 1987; BÉKÉS et al., 1987 and KÁRPÁTI et al., 1987; 1988).

1. Materials and methods

1.1. Samples

Wheat samples (cv. MV-5, MV-7, MV-8, MV-9, MV-10, MV-12, MV-08–82 and MV-12–82, each of them grown at 7 sites in Hungary: Debrecen, Eszterágpuszta, Iregszemcse, Kaposvár, Kompolt, Székkutas, Szombathely) were received by courtesy of the Hungarian Institute of Plant Growing and Qualification.

Whole wheat meals and flours were used. The milling of samples was performed on a pilot scale laboratory mill (LABOR MIM, Hungary).

1.2. Investigation of baking properties

Hagberg number, Valorigraph value, water absorption capacity and shape ratio of samples were determined using the current Hungarian Standard methods, MSZ 6369/5–70, MSZ 20501/3–78. Baking tests were performed based

on the method of MSZ 20501/3–78, as modified by MOOR (1975). Zeleny and SDS sedimentation tests were applied using the methods described by LELLEY (1973) and by PALLAGI and co-workers (1985), respectively. Milling and characterization of samples for baking properties were done in the laboratories of the Hungarian Institute of Milling.

1.3. Analytical procedures

Proteine and ash content of samples were determined using Hungarian standard methods (MSZ 5369/5–70).

1.3.1. Determination of lipid contents. To determine free (n-hexane extractable) and bound (water saturated n-butanol (WSB) extractable, followed by n-hexane extraction) wheat meal as wel las wheat flour lipids, methods of BÉKÉS (1983a) were used. All extractions were performed by repeatedly slurrying with solvents in a glass flask at room temperature, using 5 g of samples and a 10 : 1 fluid – solid ratio. The slurry was stirred for 12 hours, the liquid separated by decantation and filtration. The remaining solids were extracted a second time with a new portion of solvent for 6 hours.

The filters were combined, freed of solvent by rotary vacuum evaporator and by aeration with dry nitrogen. The dry residues were extracted with chloroform. Chloroform slurries were filtered and the chloroform removed from the filtrate by aeration under nitrogen. Lipid contents were calculated from weights. Hereafter hexane extracted and WSB-extracted lipids are referred to as free lipids (FL) and bound lipids (BL), respectively. Total lipids (TL) were calculated as the sum of FL and BL.

1.3.2. Column chromatography. FL and BL were fractionated using silicic acid column chromatography into three fractions: nonpolar lipids (NL), glycolipids (GL) and phospholipids (HL) eluted by chloroform, acetone and methanol, respectively. Polar lipids (PL) were calculated as a sum of GL and HL. Distribution of lipid fractions was determined based on the weights of dry fractions followed by freeing them from solvents by aeration with nitrogen. Details of the procedure used are given by BÉKÉS and co-workers (1983a). Flour and whole meal samples were characterized by the amounts and ratios of lipid classes, separated. Three ratios were used: FL/BL = FB, NL/PL = NP and NL/GL = = NG. Four capital-abbreviations used in the following text to characterize lipid fractions, indicate their origin (first letter: F-flour, G-whole grain), extractibility (second letter: F-free, B-bound) and class (last two letters, as shown above), see Table 20.

1.3.3. Thin-layer chromatography (TLC) was used for further fractionation and quantitative comparison of lipids. Kieselgel 60 F254 (precoated plates of Merck, No. 5715) were the adsorbent. The developing solvents were as follows (MORRISON et al., 1980): For NL to 12 cm with dietyl ether–toluene–

ethanol–acetic acid (40 : 50 : 2 : 0.2) then to 18 cm with dietyl ether –n-hexane (6 : 94); for GL to 15 cm with chloroform–acetone–acetic acid–water (10 : 90 : : 2 : 3), then to 18 cm with dietyl ether – acetic acid (99 : 1), and for HL with chloroform–methanol–ammonia (33%, w/v)–water (65 : 35 : 5 : 2.5).

1.4. Statistical analysis

The relationships between breadmaking properties and lipid analytical data of samples and their variabilities as the function of the genetical and environmental factors of wheats were studied by using different mathematical-statistical methods. These were applied as simple and multiple (stepwise) linear regressions, analysis of variance, and main component and cluster analysis, using computer programs written by the authors for COMMODORE 64 and IBM PC computers and the BMDP program package on the R32 computer of the Technical University of Budapest.

2. Results

Protein contents of whole meal and flour samples are summarized in Tables 1 and 2, respectively. Data shown are the mean values of three replicates, measured for each sample. Average C. V.-s were 3.7 and 2.9, respectively.

Table 3 shows means of loaf volume results of baking experiments, carried out in three replicates (C. V. = 8.4). Valorigraph value, Hagberg number, water absorption, shape ratio, Zeleny and SDS test results (not shown) varied widely. The minimal and maximal data, means and C. V. values are 22.5–82.8, 56.3, 27%; 204–528, 351.1, 18.5%; 52.3–69.3, 62.5, 5.4%; 1.7–2.2, 1.98, 6.4%; 3.5–6.5, 5.7, 12.2% and 1.2–5.7, 3.05, 27.2%, respectively. Both protein contents and functional properties show that there are some medium, even weak quality locations among the sites from where the samples originated. So, the

Table 1

Protein content of whole meal sample
(%)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82	X
Debrecen	13.0	13.6	13.8	12.4	12.3	12.6	12.6	13.4	12.9
Eszterágpuszta	12.4	12.6	14.7	12.6	12.3	12.3	12.4	12.5	12.7
Iregszemcse	13.1	14.2	13.8	13.5	13.7	13.1	14.1	14.2	13.7
Kaposvár	10.2	10.0	10.5	9.8	10.1	9.4	10.1	10.7	10.1
Kompolt	14.8	15.4	14.7	14.8	15.1	14.5	15.1	15.0	14.9
Székkutas	17.7	18.6	16.7	17.8	16.9	16.9	16.9	17.7	17.4
Szombathely	13.8	15.5	14.3	13.8	13.6	13.9	14.6	15.0	14.3
Average (X)	13.5	14.2	14.0	13.5	13.4	13.2	13.6	14.0	

Table 2

Protein content of flour samples
(%)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82	X
Debrecen	15.9	16.3	13.6	11.3	14.0	12.9	15.8	10.8	13.8
Eszterágpuszta	11.6	12.2	11.3	10.0	8.9	9.2	12.9	10.8	10.9
Iregszemcse	12.7	15.1	13.9	12.5	18.1	14.7	15.8	17.0	15.0
Kaposvár	11.6	8.0	6.8	8.3	8.5	7.8	7.9	9.6	8.6
Kompolt	11.4	14.7	14.0	13.9	14.2	13.3	14.0	13.1	13.6
Székkutas	14.9	15.0	15.3	13.7	14.3	14.3	14.2	15.0	14.6
Szombathely	11.6	17.0	8.0	6.8	8.5	7.8	7.9	9.6	9.6
Average (X)	12.8	14.0	11.8	10.9	12.4	11.4	12.6	12.2	

sample set used seemed to be suitable to investigate extreme effects caused by environmental factors if they existed.

Replicates of lipid extractions indicated good reproducibilities: average C. V. for FL = 4.5% and for BL = 5.2%. The average recovery by solicic acid chromatography was: 92.0%, C. V. = 2.5%. The average deviations among mean values for the fractions separated, were: NL = 5.13, GL = 6.42, HL = = 6.96. All of the lipid data shown in Tables 4–5 and 6–15 represent means of three replicates of extractions and two replicates of chromatographic separations, respectively.

19 lipid classes were identified by thin-layer chromatography: steryl esters (SE), triglycerides (TG), diglycerides (DG), free fatty acids (FFA), monoglycerides (MG), 6—0-acyl steryl glycosides (ASG), monogalactosyl di-glycerides (MGDG), monogalactosyl monoglycerides (MGMG), digalactosyl di-glyce-

Table 3

Loaf volume-results of baking tests (LV)
(cc per 360 g flour)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82	X
Debrecen	1040	950	950	1000	1050	1010	930	960	986
Eszterágpuszta	950	1050	1000	1010	890	1000	860	950	964
Iregszemcse	1010	990	910	1020	1040	1015	930	1050	996
Kaposvár	950	800	895	935	850	890	890	890	887
Kompolt	1050	970	1000	1010	1010	1010	930	1000	997
Székkutas	1065	1160	1130	1000	1100	1060	1080	1170	1096
Szombathely	950	980	800	895	850	890	890	890	893
Average (X)	1002	986	955	981	970	982	930	987	

Table 4

Free lipid contents of flours (FFFL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	1219	1557	1338	1411	1585	1454	1036	1219
Eszterágpuszta	1126	1185	1125	1155	1289	997	1024	1011
Iregszemcse	1416	1144	1305	1197	1435	1465	1036	1082
Kaposvár	872	1164	1205	984	1046	1148	1024	1079
Kompolt	1561	1534	1608	1538	1478	1213	1415	1148
Székkutas	1156	1371	1052	1030	1305	1227	1235	1063
Szombathely	931	825	1310	900	972	896	840	886

Table 5

Bound lipid contents of flours (FFBL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	970	1000	870	950	950	890	670	900
Eszterágpuszta	1170	1220	1020	1100	1055	1015	1050	1090
Iregszemcse	680	580	640	740	700	550	670	750
Kaposvár	1190	1150	920	940	910	1100	1040	1130
Kompolt	1120	990	910	980	1070	940	1050	1050
Székkutas	710	740	1150	830	690	820	820	780
Szombathely	1190	1190	1150	960	940	1110	1080	1130

Table 6

Free lipid content of whole meals (GGFL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	1887	2067	2571	2226	2171	2432	2200	2378
Eszterágpuszta	2021	1883	2695	1978	1965	1986	1998	2181
Iregszemcse	2551	2349	2600	2395	2442	2534	2460	2932
Kaposvár	1889	1770	1937	1891	1857	1926	1735	1837
Kompolt	2056	2259	2695	2193	2065	2203	2146	2682
Székkutas	2451	2674	2620	1845	2016	1834	1707	1859
Szombathely	2076	2099	2665	2436	2358	2335	2159	2047

Table 7

Free nonpolar lipid contents of flours (FFNL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	841	1095	934	967	1097	1013	736	838
Eszterágpuszta	774	823	774	784	862	672	723	699
Iregszemcse	972	796	914	813	1011	1022	736	763
Kaposvár	592	780	777	676	718	752	685	736
Kompolt	1062	1079	1121	1066	1038	854	998	814
Székkutas	783	920	728	701	902	849	860	726
Szombathely	648	593	877	591	647	589	551	601

Table 8

Free glycolipid contents of flours (FFGL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	129	170	151	173	188	181	114	143
Eszterágpuszta	115	141	131	143	152	122	110	111
Iregszemcse	168	124	148	130	158	164	114	114
Kaposvár	93	133	159	129	130	137	120	120
Kompolt	179	166	177	167	166	134	154	124
Székkutas	125	145	114	111	143	134	137	116
Szombathely	107	81	149	108	111	106	98	101

Table 9

Free phospholipid contents of flours (FFHL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	249	292	254	272	299	260	187	239
Eszterágpuszta	238	221	220	228	275	203	191	201
Iregszemcse	277	225	244	255	267	280	187	206
Kaposvár	187	251	270	179	198	260	219	223
Kompolt	320	290	309	305	274	225	264	209
Székkutas	249	306	210	218	260	245	239	223
Szombathely	176	151	284	202	215	200	191	185

Table 10

Bound nonpolar lipid contents of flours (FBNL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	103	166	104	137	112	156	84	142
Eszterágpuszta	183	190	190	202	180	188	180	132
Iregszemcse	115	93	115	89	95	85	84	104
Kaposvár	207	208	118	141	130	162	167	156
Kompolt	204	175	107	147	206	153	198	192
Székkutas	105	141	167	155	128	161	199	147
Szombathely	207	206	208	153	152	189	173	140

Table 11

Bound glycolipid contents of flours (FBGL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	329	340	260	277	311	242	187	262
Eszterágpuszta	344	361	289	330	325	278	308	336
Iregszemcse	215	159	182	219	227	152	187	245
Kaposvár	343	375	347	271	318	335	296	331
Kompolt	310	275	270	300	312	263	289	294
Székkutas	214	203	351	224	193	232	210	216
Szombathely	343	343	375	379	358	324	309	339

Table 12

Bound phospholipid contents of flours (FBHL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	643	655	506	537	527	492	400	495
Eszterágpuszta	642	700	541	569	550	549	561	622
Iregszemcse	350	327	343	432	378	313	399	401
Kaposvár	640	567	454	528	462	603	577	643
Kompolt	606	540	533	534	552	524	563	564
Székkutas	391	396	632	451	369	427	411	417
Szombathely	640	640	567	428	430	597	598	651

Table 13

Free nonpolar lipid contents of whole meals (GFNL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	1504	1706	2141	1801	1729	1972	1803	1986
Eszterágpuszta	1643	1499	2247	1588	1617	1589	1656	1784
Iregszemcse	2084	1945	2180	1955	1991	2069	2054	2423
Kaposvár	1476	1440	1552	1483	1492	1515	1374	1472
Kompolt	1682	1900	2247	1816	1710	1819	1806	2243
Székkutas	2073	2242	2171	1584	1677	1537	1423	1542
Szombathely	1716	1765	2277	2028	1983	1955	1817	1733

Table 14

Free glycolipid contents of whole meals (GFGL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	111	101	127	126	123	132	103	114
Eszterágpuszta	104	106	130	107	100	102	94	110
Iregszemcse	130	109	121	123	127	131	115	140
Kaposvár	114	89	105	110	97	111	101	100
Kompolt	102	96	130	105	93	105	94	131
Székkutas	112	121	124	81	99	87	83	94
Szombathely	93	101	101	92	98	103	98	92

Table 15

Free phospholipid contents of whole meals (GFHL)
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
Debrecen	272	259	303	298	319	328	293	296
Eszterágpuszta	274	278	318	284	247	295	248	288
Iregszemcse	338	295	299	317	324	334	291	369
Kaposvár	299	240	280	298	268	300	260	266
Kompolt	272	264	318	273	262	279	246	308
Székkutas	266	310	325	181	239	210	201	223
Szombathely	267	233	287	316	277	277	244	222

Table 16

Lipid compositions of whole meal samples from Eszterágpuszta
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
				Free lipids				
SE	20.2	16.1	30.2	23.6	22.5	24.8	24.4	32.8
TG	342.9	247.8	280.7	302.0	309.3	232.9	355.9	271.1
DG	82.2	102.5	93.7	81.2	81.2	86.3	84.9	94.3
FFA	45.5	48.3	44.4	43.8	43.3	45.6	47.3	39.8
MG	16.6	18.6	15.7	20.6	19.1	23.6	21.2	25.4
ASG	13.1	11.8	12.7	10.6	13.1	16.1	15.6	13.6
MGDG	8.2	4.1	7.6	5.2	11.2	8.28	10.4	9.6
MGMG	2.2	5.8	2.7	2.7	3.3	3.7	4.9	4.3
DGDG	13.8	9.9	10.6	11.1	16.0	13.0	14.1	21.1
DGMG	.8	1.3	1.4	.9	.3	.7	1.2	.7
NAPE	7.7	8.3	8.6	9.5	10.6	10.7	14.0	12.1
NALPE	3.9	3.7	4.5	4.9	5.5	5.2	7.3	5. 7
PE+PG	5.4	3.1	4.8	3.1	6.8	5.2	2.2	3.9
PC	25.3	19.8	20.4	17.4	11.5	15.2	29.2	18.9
PI				in traces				
LPC	12.2	11.2	10.9	11.7	10.8	12.5	14.0	11.8
LPE+LPI	6.9	7.3	8.1	6.9	8.3	6.9	7.8	8.9
				Bound lipids				
SE	6.1	3.3	7.2	4.3	7.6	9.4	10.5	5.9
TG	55.5	56.3	49.9	50.6	50.8	50.0	59.1	56.5
DG	60.1	64.3	66.9	66.7	72.3	75.6	65.9	60.5
FFA	7.1	8.3	8.4	5.8	7.0	8.3	8.9	6.3
MG	29.4	31.1	29.7	29.7	28.6	31.9	30.7	30.5
ASG	14.1	17.2	15.7	14.1	14.3	13.8	14.5	13.5
MGDG	35.1	42.9	42.6	48.4	44.7	47.2	46.3	40.8
MGMG	55.9	55.7	62.7	76.2	78.4	72.6	45.2	44.1
DGDG	159.3	125.8	92.6	110.6	89.8	71.2	106.2	71.1
DGMG	21.3	16.6	21.4	18.4	19.8	27.3	25.8	19.1
NAPE	21.1	25.5	25.3	24.5	25.6	19.4	22.7	19.7
NALPE	22.2	19.9	23.5	22.8	19.5	17.9	24.3	19.8
PE+PG	17.1	9.2	14.2	10.5	12.6	8.2	14.8	8.2
PC	84.0	49.9	74.4	52.0	60.0	53.8	33.3	47.7
PI	15.4	12.0	12.8	12.7	13.0	14.0	17.8	12.9
LPC	40.1	35.5	33.0	39.7	47.4	40.7	47.6	33.1
LPE+LPI	15.6	10.6	16.1	16.7	20.1	12.9	26.5	21.3

Table 17

Lipid compositions of flour samples from Eszterágpuszta
(mg per 100 g)

	MV 5	MV 7	MV 8	MV 9	MV 10	MV 12	MV 08–82	MV 12–82
				Free lipids				
SE	28.5	16.7	24.0	29.9	17.1	21.6	24.7	25.8
TG	250.5	195.4	161.9	161.8	167.2	190.7	177.3	173.9
DG	63.8	60.9	68.8	55.1	55.4	66.7	81.8	72.5
FFA	33.9	35.2	36.6	31.9	31.5	43.1	43.6	32.6
MG	12.0	16.1	15.2	19.5	16.6	22.8	11.1	22.8
ASG	7.6	6.9	7.7	7.3	6.9	9.6	6.2	6.1
MGDG	6.2	8.8	6.6	4.2	8.2	7.2	8.4	7.6
MGMG	10.2	11.1	9.2	7.3	8.5	5.1	7.7	3.2
DGDG	31.8	28.4	29.7	30.3	24.7	32.0	31.8	27.1
DGMG	.6	1.3	1.1	.2	.1	.9	1.2	.7
NAPE	10.9	15.4	9.9	5.7	6.0	5.5	6.5	10.1
NALPE	15.3	8.9	5.1	6.8	5.5	6.5	7.3	10.4
PE+PG	7.8	4.9	7.7	7.5	8.6	6.2	5.9	6.9
PC	17.9	10.5	13.6	13.8	8.1	10.2	7.6	6.3
PI				in traces				
LPC	7.6	6.1	7.9	8.7	6.8	5.5	4.9	4.8
LPE+LPI	4.9	5.1	5.5	4.9	5.1	4.2	3.8	3.1
				Bound lipids				
SE	1.5	1.2	1.1	1.3	1.2	1.7	1.6	1.9
TG	34.5	33.1	44.0	49.7	32.6	35.7	45.9	35.7
DG	13.9	19.6	14.3	21.0	17.2	12.5	12.0	12.0
FFA	4.8	4.9	3.9	4.3	6.3	5.8	3.4	5.3
MG	18.2	15.5	21.5	29.3	18.9	28.6	21.2	22.8
ASG	5.2	5.7	6.6	8.2	6.8	8.2	6.9	4.2
MGDG	28.7	30.4	33.3	43.0	28.8	39.2	45.7	37.7
MGMG	29.4	25.6	29.5	40.3	31.1	39.1	38.7	38.4
DGDG	72.8	85.3	75.2	87.7	74.1	83.1	73.4	92.4
DGMG	15.8	10.3	10.3	16.2	14.9	11.0	19.1	17.2
NAPE	38.9	38.8	30.0	27.3	27.6	26.3	22.7	30.1
NALPE	24.8	22.1	24.6	27.6	23.9	30.1	30.4	31.9
PE+PG	5.5	7.2	4.7	5.4	7.4	9.6	8.1	9.9
PC	32.2	31.9	30.3	25.2	30.8	44.6	46.0	31.6
PI	8.4	9.5	11.6	9.4	8.3	13.6	9.3	6.6
LPC	10.1	12.5	13.0	18.7	14.9	15.0	21.1	15.6
LPE+LPI	6.9	7.3	8.1	6.9	8.3	6.9	7.8	8.9

Table 18

Lipid compositions of whole meal samples of variety MV 7, grown at different locations

(mg per 100 g)

	A	B	C	D	E	F	G
			Free lipids				
SE	16.4	16.1	15.6	14.0	17.9	10.2	13.4
TG	191.3	247.8	197.2	190.8	276.5	153.7	155.3
DG	192.6	102.5	163.1	156.4	176.3	153.7	184.7
FFA	38.9	48.3	43.3	48.7	49.0	35.6	35.4
MG	14.0	18.6	15.5	10.2	9.8	11.5	11.7
ASG	13.8	11.8	13.3	14.0	12.4	13.2	13.6
MGDG	3.8	4.1	2.6	4.2	1.2	5.3	3.4
MGMG	11.4	5.8	12.8	10.8	13.5	10.4	7.9
DGDG	18.8	9.9	10.6	16.0	11.4	17.6	11.1
DGMG	.7	1.3	.6	1.3	.6	.5	.7
NAPE	5.2	8.3	21.0	17.6	10.4	10.7	9.2
NALPE	5.7	3.7	5.1	6.4	3.7	7.9	7.3
PE+PG	3.3	3.1	4.3	5.1	2.8	5.0	4.2
PC	23.4	19.8	22.2	25.5	22.7	23.1	24.2
PI			in traces				
LPC	11.3	11.2	14.7	10.2	13.2	11.7	10.9
LPE+LPI	6.3	7.3	5.4	7.4	7.2	5.6	6.8
			Bound lipids				
SE	9.8	3.3	3.4	5.2	8.9	8.0	4.4
TG	50.9	56.3	39.3	56.7	67.5	50.0	59.3
DG	63.4	64.3	62.2	65.1	48.4	32.4	55.5
FFA	9.1	8.3	4.4	7.8	12.0	8.7	7.3
MG	28.4	31.1	9.8	12.9	18.6	15.4	18.9
ASG	10.1	17.2	15.7	16.9	19.2	17.6	15.6
MGDG	28.2	42.9	29.4	43.5	30.8	23.1	30.4
MGMG	48.2	55.7	55.9	79.3	101.0	94.7	64.9
DGDG	73.8	125.8	81.5	80.6	119.6	182.1	114.7
DGMG	11.8	16.6	11.2	22.2	15.3	9.7	11.2
NAPE	21.5	25.5	11.3	30.8	19.2	12.3	13.6
NALPE	16.7	19.9	12.9	18.8	12.8	16.4	15.6
PE+PG	7.4	9.2	6.6	7.55	8.9	9.7	7.4
PC	55.4	49.9	55.9	58.1	60.9	55.5	49.2
PI	13.1	12.0	15.6	13.9	15.1	12.1	12.8
LPC	35.2	35.5	47.9	32.7	51.1	39.6	44.2
LPE+LPI	8.1	10.6	6.9	12.7	9.7	10.0	9.9

A: Kaposvár B: Eszterágpuszta C: Iregszemcse
D: Székkutas E: Debrecen F: Kompolt
G: Szombathely

Table 19

Lipid compositions of flour samples of variety MV 7, grown at different locations
(mg per 100 g)

	A	B	C	D	E	F	G
			Free lipids				
SE	15.0	16.7	10.9	11.6	11.9	13.1	14.7
TG	190.0	195.4	162.6	171.4	241.2	233.4	155.9
DG	51.9	60.9	67.5	68.9	43.9	57.1	54.2
FFA	28.8	35.2	23.9	43.3	21.9	23.6	37.6
MG	19.8	16.1	18.5	16.7	15.4	14.9	17.2
ASG	5.1	6.9	4.9	6.6	5.9	4.8	5.6
MGDG	24.6	8.8	10.9	13.9	16.6	16.1	17.4
MGMG	15.4	11.1	14.7	11.0	14.2	17.6	14.3
DGDG	45.3	28.4	29.8	27.8	33.4	22.3	25.9
DGMG	.7	1.3	2.1	1.9	1.3	1.5	1.2
NAPE	18.4	15.4	13.7	14.5	18.6	13.4	14.3
NALPE	12.8	8.9	15.5	11.9	10.5	9.2	11.3
PE+PG	3.1	4.9	3.8	4.8	4.7	2.9	4.1
PC	12.2	10.5	9.3	14.9	18.3	11.6	12.2
PI			in traces				
LPC	3.1	6.1	3.4	8.1	7.3	4.8	4.0
LPE+LPI	4.2	5.1	3.8	2.5	3.0	4.4	5.0
			Bound lipids				
SE	1.6	1.2	1.9	3.6	1.2	3.4	2.4
TG	11.2	33.1	26.6	50.4	28.7	36.8	32.1
DG	14.9	19.6	20.2	29.9	36.2	28.6	24.5
FFA	10.9	4.9	5.4	13.4	10.3	7.4	7.7
MG	20.4	15.5	11.7	20.6	15.3	17.8	18.2
ASG	8.0	5.7	4.6	7.8	7.6	5.3	5.6
MGDG	22.7	30.4	21.8	32.4	30.9	26.3	21.4
MGMG	41.1	25.6	22.8	55.6	38.3	28.7	34.9
DGMG	8.6	10.3	9.6	13.6	9.7	10.6	12.1
DGDG	49.2	85.3	67.7	102.7	85.0	66.9	71.2
NAPE	26.6	38.8	40.8	44.5	45.1	32.5	34.8
NALPE	18.6	22.1	15.9	23.1	20.4	20.2	21.3
PE+PG	3.8	7.2	4.8	8.3	8.2	7.2	5.1
PC	20.1	31.9	26.8	27.4	31.5	27.2	29.5
PI	7.2	9.5	6.0	6.1	6.9	8.0	6.9
LPC	18.5	12.5	9.3	21.1	19.6	15.9	14.0
LPE+LPI	4.6	7.3	5.1	8.0	7.6	8.4	7.8

A: Kaposvár B: Eszterágpuszta C: Iregszemcse
D: Székkutas E: Debrecen F: Kompolt
G: Szombathely

rides (DGDG), digalactosyl monoglycerides (DGMG), N-acyl phosphatidyl ethanol amines (NAPE), N-acyl lysophosphatidyl ethanol amines (NALPE), phosphatidyl ethanolamines (PE), phosphatidyl glycerols (PG), phosphatidyl cholines (PC), phosphatidyl inositols (PI), lysophosphatidyl ethanolamines (LPE), lysophosphatidyl choline (LPC) and lysophosphatidyl inositols (LPI), from which PE + PG and LPE + LPI were quantified together because of their poor resolutions. Lipid compositions of flours and whole meals of samples from Eszterágpuszta and of variety MV 7 from different locations are presented in Tables 16–17 and 18–19, respectively.

3. Discussion

3.1. Intervariety and environmental effects on baking quality and on lipid composition results

3.1.1. Effects on baking quality. Laboratory milling procedure gave uniform milling yields: yields varied between 68.3–73.4% (mean: 71.2, C. V.: 3.2%). The same data for whole meal protein content (Table 1) are 9.4–18.6%, 13.7%, 15.3%. Average C. V. was much higher among varieties than among locations (2.5% and 18.3%, respectively).

Whole meal protein contents of samples from Kaposvár, as most of the other parameters measured, differed significantly from the data of samples, originating from other locations (mean = 10.1, C. V. = 4.0). Among varieties, MV 12–82 differed significantly from the others. Average protein contents of flour samples (Table 2) did not show significant differences among varieties but the location-dependency is remarkable.

The average loaf volume in the whole sample set is 974 cc/360 g, (C. V. = = 8.4%). Loaf volume data (Table 3) showed similar variations between locations and varieties: significant difference was not found among varieties while significant differences were observed ($P = 0.025$) among sites. Each functional property investigated varied much more among locations than among varieties. Results of samples from Kaposvár always showed significant negative differences while results of samples from Székkutas were significantly stronger than the rest ($P = 0.01$). On the other hand, significant deviations among varieties were found only in a few cases ($P = 0.5$, only), where MV 82–12 showed better and MV 8 showed weaker functional properties. Thus, the deviation caused by differences in protein quality is lower than the one caused by differences in protein contents. However, loaf volume potential (LV/protein content) data showed significantly higher results for MV 12–82.

3.1.2. Effects on lipid content and composition. Strong effect of environmental factors were found among lipid data (Tables 4–15), too. Significance level

of differences among varieties was only P = 1.0–5.0, while the site-effect was much stronger (significant differences at P = 0.1–1.0).

As it was found in Canadian varieties (BÉKÉS et al., 1986), environmental differences resulted in different levels of variations in different lipid classes: F-values in analysis of variance were ten times higher for FL than for BL in both flours and whole meals. In whole meals, the relative sequence of sensitivity from environment was NL $\gg$ GL $>$ HL, in both FL and BL. The trend is similar in the flour lipids, as well. However, different proportions of flour NL was found in flour, average: 54.4%, C. V. = 10.3%, the latter one is remarkably high, taking into consideration that milling yields were almost uniform.

Comparing the compositions of FL and BL, NL/PL ratios were found to be the most characteristic parameters: they vary between 2 and 3 for FL, while less than 0.3 for BL. HL values were found to be generally double those of GL from the same sample. This finding is not in agreement with other data published about lipid composition of North American (CHUNG & TSEN, 1975) and European wheat flours (HARGIN & MORRISON, 1980). where approximately 1 : 1 GL/HL were found. The authors have no explanation for these increased phospholipid levels.

Comparison of whole meal and flour lipid data showed quite low relationships: r(FL) = 0.229, r(NL) = 0.230, r(GL) = 0.320, r(PL) = 0.370, but in agreement with other investigations (BÉKÉS et al., 1986., MORRISON, 1983) polar lipids — having mainly structure functions in the seed — showed significantly higher relationships than nonpolar lipids.

TG (162–356 mg per 100 g dry matter content) was found as the main component of FFNL of samples from Eszterágpuszta, shown in Tables 16 and 17. The wide distributions of apolar components are characteristical among varieties however the sequence of relative amounts is the same: TG $>$ DG $>$ $>$ FFA $>$ MG $>$ SE $>$ ASG. TG is the main component in FBNL of samples, too, however its contribution is much lower than in FFNL (the average TG/DG ratios for example: FFNL = 3.92, FBNL = 2.48). Generally double amount of ASG and 1.5 times more TG were found in GFNL-s than in FFNL-s. Component-ratios were very similar in both free and bound lipids in whole meals, differences among varieties were much lower than the ones in flour lipid fractions. These results emphasize that milling action is a key factor to cause variation of lipid distribution even at uniform milling yields.

In GL fraction DGDG $>$ MGDG $>$ MGMG $>$ DGMG was the sequence of relative amounts in each variety. PC was the main component in HL fractions, where the phosphatide/lysophosphatide ratio was found as the most characteristical difference among varieties. Although, these differences might indicate the different activities of phospholipases they may also indicate different levels of starch-degradations among the samples (LUKOW et al., 1985).

Lipid composition data of MV7 samples from different locations (Tables 18 and 19) showed that only quantitative differences can be found, the sequence of the relative amounts is identical in each sample. These quantitative differences are higher among flours than in whole meals, on the other hand, they are higher in free than in bound lipids. TG was found to be the most variable component in each case.

Variations of lipid content and composition found in this study seem to be caused mainly by environmental factors. Of course, it has to be emphasized that wheat varieties investigated here are genetically closely related. In a sample population with wide genetical background more marked differences could exist, as it was found in a smaller experimental design, investigating different European varieties, grown in Hungary (SIMON-SARKADY & BÉKÉS, 1987).

It is remarkable, that in agreement with earlier observations on Canadian varieties (BÉKÉS et al., 1986), ratios of nonpolar and polar lipid fractions, significantly dependent on environmental factors, individually, (FFNP, FFNG, GFNP, GFNG) do not show any effect of site.

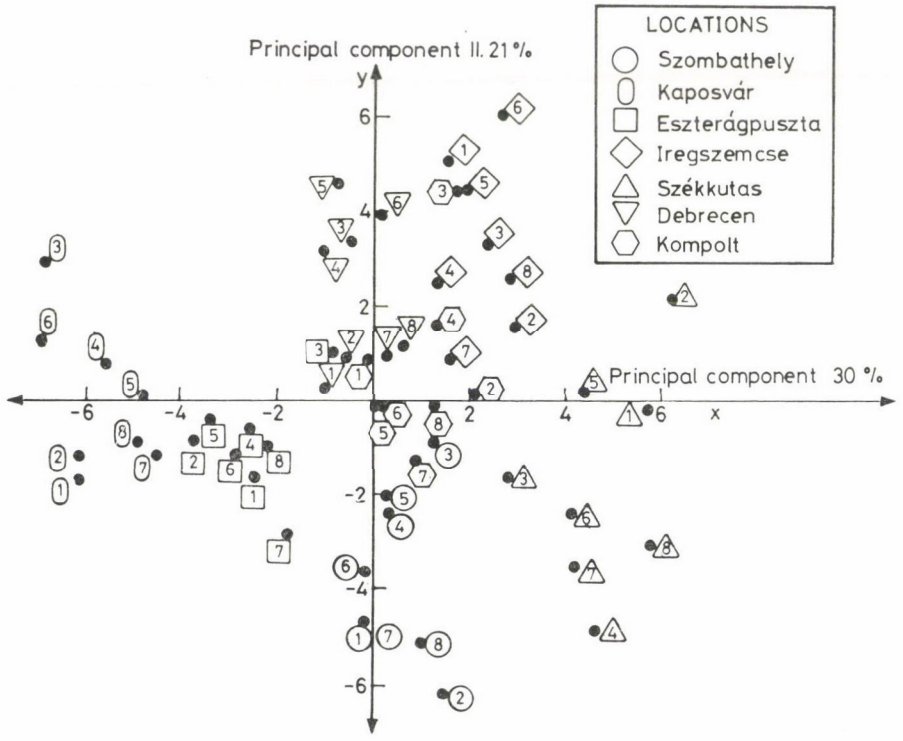


Fig. 1. Mapping of wheats by principal components analysis in the plane axis 1-axis 2. The first and second principal components were calculated from lipid data and baking quality. Varieties: 1-MV-5; 2-MV-7; 3-MV-8; 4-MV-9⁹ 5-MV-10; 6-MV-12; 7-MV-08-82; 8-MV-12-82

3.1.3. Main component and cluster analysis. Investigating the topology of samples in the sample population (n = 56), the usual Euclidian distances — calculated from the differences of main component coordinates — were used as suitable measure to distinguish certain classes. The localisation of samples in the plan of the first two main components (I and II) is shown in Fig. 1.

Components I and II contained 30% and 21% of the information, respectively. Dendrogram of samples, presented in Fig. 2 shows that the samples are classified by their growing sites, similarity of samples from the same sites is between 85–95%. Projections of the axes of original data, fallen onto the plan expended by components I and II are shown in Fig. 3. One group of functional properties (water absorption, Hagberg number, shape ratio) was divided between component I and II, while the rest located in the positive direction of I. Bound lipids located between component I and II, free lipids — as another class — can be found in the direction of I. From the point of view of possible relationships between functional properties and lipid parameters it is important that one class of functional properties including loaf volume was located very close to certain lipid parameters, namely FFNP, FFNG, GFNP and GFNG.

The calculations were repeated with data normalised to 1% protein contents (Figs. 4–6). Comparisons of localisations of corresponding data points show that the main difference caused by normalisation is the shifts of functional

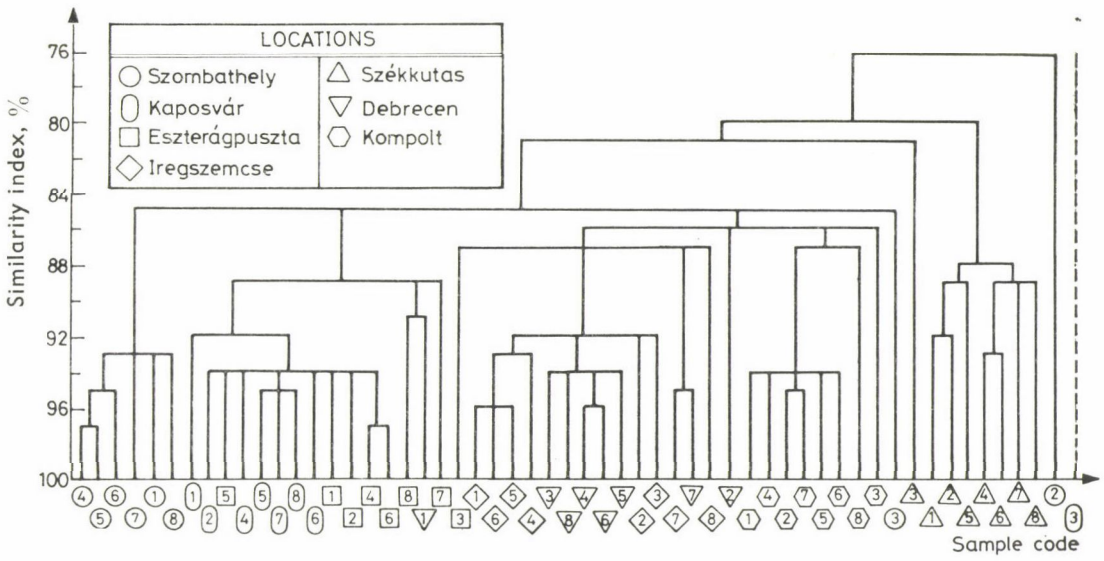


Fig. 2. The similarity dendrogram of wheats based on lipid data and data characteristics of baking quality. Varieties: 1-MV-5; 2-MV-7; 3-MV-8; 4-MV-9; 5-MV-10; 6-MV-12; 7-MV-08-82; 8-MV-12-82

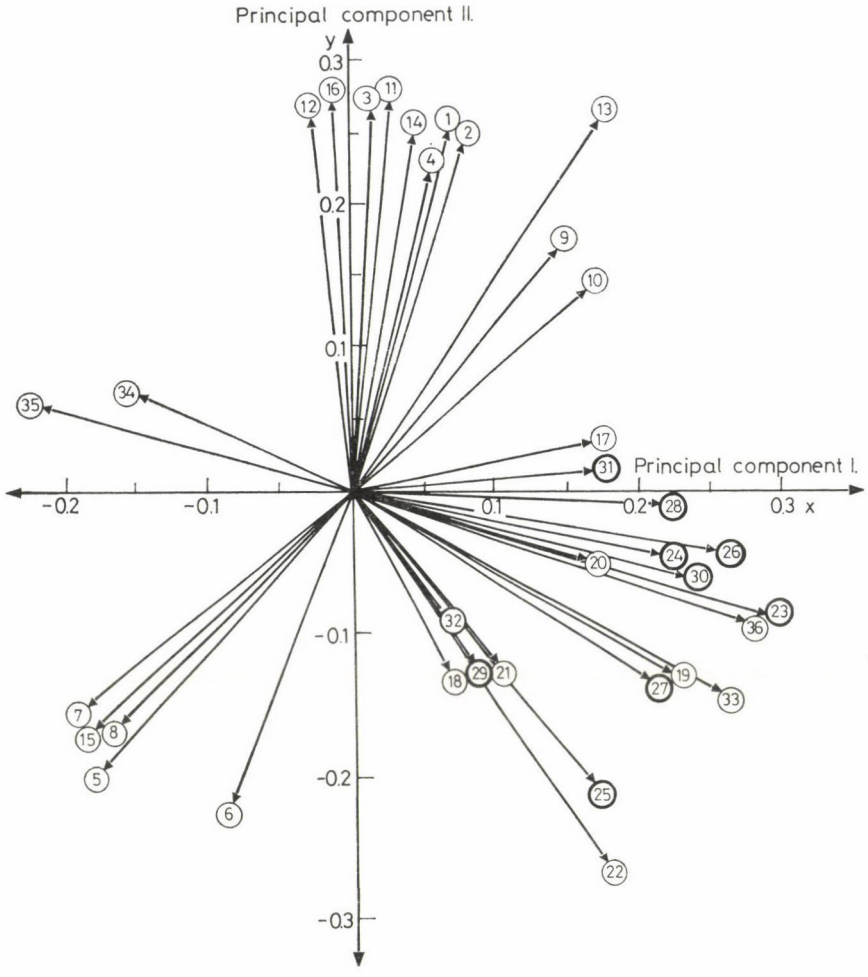


Fig. 3. Projection of the original characteristics to the plane of axes of the first and second principal components calculated from lipid data and baking quality. (For codes see Tables 20 and 20/a)

parameters from the positive direction of main component to the negative one. Lipid parameters describing polarity ratios are located here, indicating close correlations with functional parameters.

3.2. Relationships between baking properties and lipid data

Single linear regression study between baking properties and lipid data showed low correlation. First column of Table 21 shows r values for loaf volume with different lipid parameters.

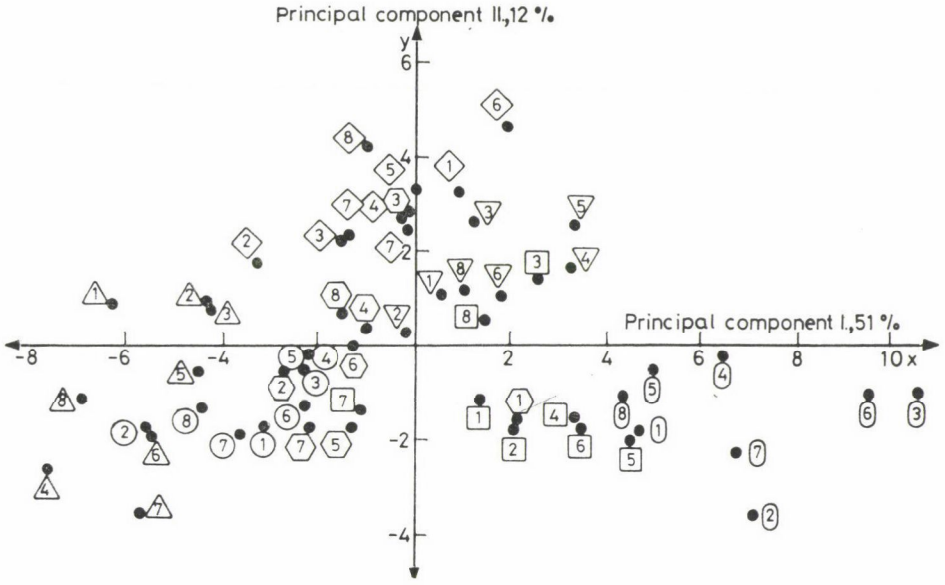


Fig. 4. Mapping of wheats by principal components analysis in the plane axis 1-axis 2. The first and second principal components were calculated from normalized lipid data and baking quality (For codes see Fig. 1.)

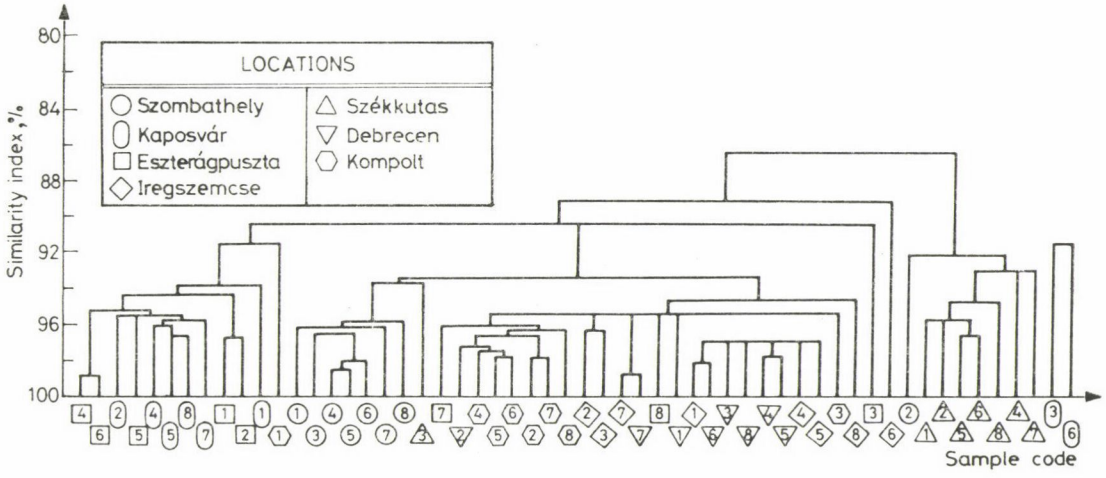


Fig. 5. The similarity dendrogram of wheats based on normalized lipid data and data characteristics of baking quality. (For codes see Fig. 1.)

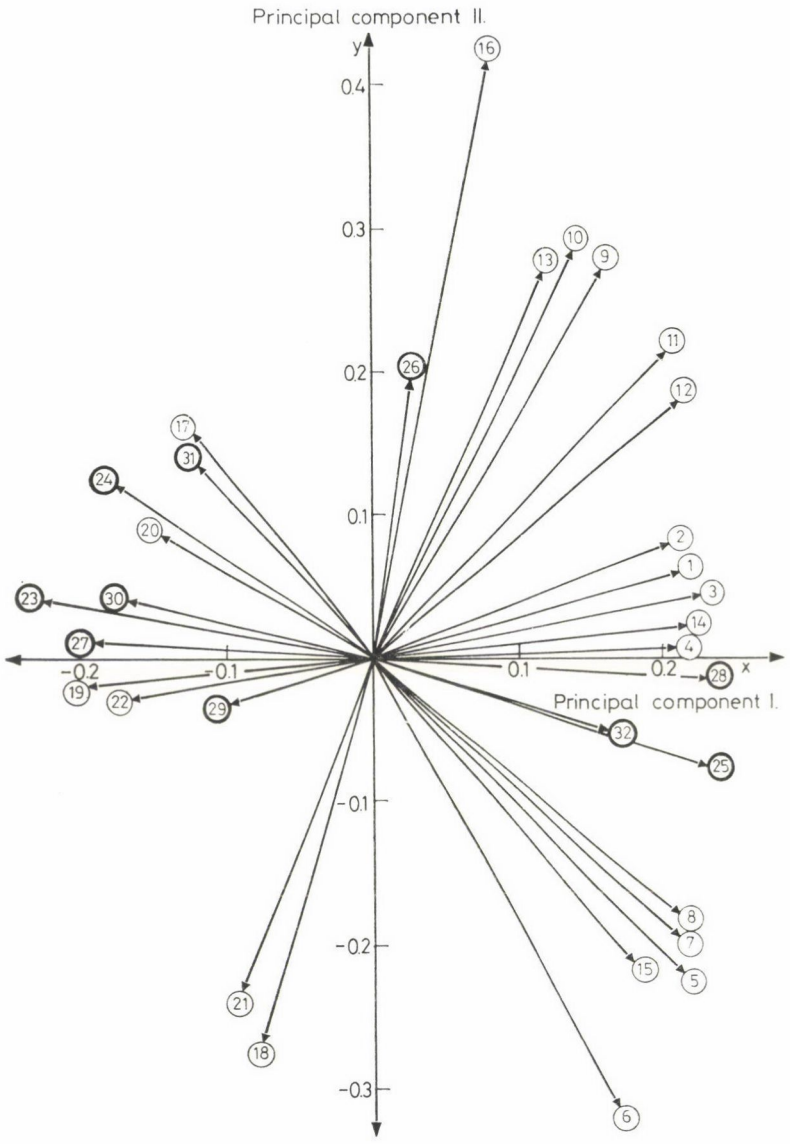


Fig. 6. Projection of the normalized data to the plane of axes of the first and second principal components calculated from lipid data and baking quality. (For codes see Tables 20 and 20/a)

Based on the results on Canadian wheat samples (BÉKÉS et al., 1986), applying the information from main component and cluster analysis in this study, calculations were repeated with data, normalised to 1% protein content. In this case significant relationships can be found for each quality parameter ($P < 0.05$). Linear correlation coefficients for normalised loaf volumes are

Table 20

Symbols of lipids

Symbols	Property	Code number
FFFL	flour free total lipid	1
FFNL	flour free nonpolar lipid	2
FFGL	flour free glycolipid	3
FFHL	flour free phospholipid	4
FFBL	flour bound total lipid	5
FBNL	flour bound nonpolar lipid	6
FBGL	flour bound glycolipid	7
FBHL	flour bound phospholipid	8
GFFL	grain free total lipid	9
GFNL	grain free nonpolar lipid	10
GFGL	grain free glycolipid	11
GFHL	grain free phospholipid	12
FFBB	FFFL/FFBL	13
FFPL	flour free polar lipid (FFGL + FFHL)	14
FBPL	flour bound polar lipid (FBGL + FBHL)	15
GFPL	grain free polar lipid (GFGL + GFHL)	16
FFNP	FFNL/FFPL	17
FBNP	FBNL/FBPL	18
GFNP	GFNL/GFPL	19
FFNG	FFNL/FFGL	20
FBNG	FBNL/FBGL	21
GFNG	GFNL/GFGL	22

Table 20/a

Determined properties

Property	Code number
Wet gluten content	23
Spreading of wet gluten	24
Water absorption	25
Valorigraph value	26
Falling number of Hagberg	27
Loaf volume	28
Form ratio of loaf	29
Sedimentation test in SDS	30
Sedimentation test of Zeleny	31
Flour moisture content	32
Flour protein content	33
Whole grain moisture content	34
Whole grain total lipid content	35
Whole grain protein content	36

Table 21

Linear correlation coefficients between loaf volume and lipid contents, calculated from measured and normalized data (n = 56)
(Normalization — signed with "c" — was calculated onto 1% of protein content in flours or whole meals.)

LV		LVc	
FFFL	.102	FFFLc	.565
FFNL	.117	FFNLc	.504
FFGL	.047	FFGLc	.623
FFHL	.067	FFHLc	.658
FFPL	.062	FFPLc	.659
FFBB	—.208	FFBB	.253
FFNP	—.245	FFNP	—.823
FFNG	—.119	FFNG	—.723
FFBL	.115	FFBLc	.771
FBNL	.036	FBNLc	.644
FBGL	.225	FBGLc	.776
FBHL	.069	FBHLc	.698
FBPL	.129	FBPLc	.758
FBNP	.026	FBNP	—.002
FBNG	.082	FBNG	—.014
GFFL	.025	GFFLc	.289
GFNL	.022	GFNLc	.171
GFGL	.089	GFGLc	.714
GFHL	.013	GFHLc	.722
GFPL	.029	GFPLc	.733
GFNP	.032	GFNP	—.940
GFNG	.024	GFNG	—.782

shown in the second column of Table 21. For other quality parameters, the highest r values were: Valorigraph value: 0.361 (FFBB); Hagberg number: 0.701 (FFHLc); water absorption: 0.865 (FFBLc); Zeleny test: 0.491 (FBGLc); SDS test: 0.644 (FBGLc). These data indicate that mostly polar lipids are related to functional properties but while free polar lipids (mainly GL) and the NL/PL or NL/GL ratios are important for loaf volume, the bound polar lipids seem to be more important for other parameters. Similarly to the results from Canadium varieties (BÉKÉS et al., 1986), mixing properties did not show strong correlation with free lipids as was found by CHUNG and co-workers (1982) in U. S. varieties.

Multiple (stepwise) linear regression resulted in very good relationships to predict loaf volume:

$$\text{LVc} = 37.62 + 0.81 * \text{FFGL} - 1.57 * \text{FFNP} \qquad r = 0.953$$
$$\text{LVc} = 43.70 + 2.69 * \text{GFGL} - 1.27 * \text{GFNP} \qquad r = 0.944$$

In agreement with other studies carried out on completely different sample sources, namely on Canadian (BÉKÉS et al., 1986) and on Australian varieties (PANOZZO et al., 1988) protein content, free glycolipids and the ratio of free nonpolar and polar lipids are the three parameters with which loaf volume can be predicted. This finding underlines the importance of free lipids (their amounts and polarity distribution) in relation to baking properties.

Multiple linear equations using these parameters seem to be suitable for predicting loaf volume with less than 10% relative error. This precisity might be satisfactory for application in breeding tests to select for quality.

Literature

BELL, B. M., DANIELS, D. G. H., FEARN, T. & STEWART, B. A. (1987): Lipid composition, baking quality and other characteristics of wheat varieties grown in the U. K. *J. Cereal Sci., 5.* 277–286.

BÉKÉS, F., KÁRPÁTI, M., ÖRSI, F., SMIED, I. & LÁSZTITY R. (1987): A búzalipidek valamint a fehérje-lipid komplexek hatása a sütőipari minőségre. III. (The effects of wheat lipids and their protein-lipid complexes on baking properties. Part III.) *Sütőipar, 34,* 203–205.

BÉKÉS, F. & MACRITCHIE, F. (1988a): The effects of lipids and protein–lipid interactions on the functional properties of wheat flour. *Proc. Int. Sem. Functional Properties of Food Proteins.* MÉTE, Budapest, pp. 48–57.

BÉKÉS, F. & MACRITCHIE, F. (1988b): Functional properties of lipid binding proteins of wheat endosperm. *Proc. 34. Austr. Cer. Chem. Conf.* Sydney, pp. 1041–1042.

BÉKÉS, F., ZAWISTOWSKA, U. & BUSHUK, W. (1983a): Protein-lipid complexes in the gliadin fraction. *Cereal Chem., 60,* 371–378.

BÉKÉS, F., ZAWISTOWSKA, U. & BUSHUK, W. (1983b): Lipid mediated aggregation of gliadin. *Cereal Chem., 60,* 379–380.

BÉKÉS, F., ZAWISTOWSKA, U., ZILLMAN, R. R. & BUSHUK, W. (1983c): Prediction of breadmaking quality of wheat cultivars from lipid content and composition. *Cer. Fd World, 28,* 565.

BÉKÉS, F., ZAWISTOWSKA, U., ZILLMAN, R. R. & BUSHUK, W. (1986): Relationship between lipid content and composition and loaf volume of 26 common spring wheats. *Cereal Chem., 63,* 327–331.

BUSHUK, W., BÉKÉS, F., MACMASTER, G. & ZAWISTOWSKA, U. (1985a): Carbohydrate and lipid complexes with gliadin and glutenin. – In: GRAVELAND, A. & MOONEN, J. H. E. (Eds.) *Proc. II. Workshop on Gluten Proteins,* Wageningen, 1984. pp. 27–39.

BUSHUK, W., BÉKÉS, F., MACMASTER, G. & ZAWISTOWSKA, U. (1985b): Kohlenhydrate und Lipidkomplexe mit Proteinen im Kleber. *Getreide Mehl Brot, 17,* 35–37.

CHIU, C. M. & POMERANZ, Y. (1966): Changes in extractibility of lipids during breadmaking. *J. Fd Sci., 31,* 753–758.

CHUNG, O. K., POMERANZ, A. & FINNEY, K. (1982): Relation of polar lipid content to mixing requirement and loaf volume potential of hard red winter wheat flour. *Cereal Chem., 59,* 14–20.

CHUNG, O. K. & TSEN, C. C. (1975a): Changes in lipid binding and protein distribution during dough mixing. *Cereal Chem., 52,* 533–540.

HARGIN, K. D. & MORRISON, W. R. (1980): The distribution of acyl lipids in the germ, aleurone, starch and non-starch endosperm of four wheat varieties. *J. Sci. Fd Agric., 31,* 877–888.

KÁRPÁTI, M. BÉKÉS, F., LÁSZTITY, R. & SMIED, I. (1987): A búzalipidek valamint a fehérje-lipid komplexek hatása a sütőipari minőségre. II. (The effects of wheat lipids and their protein-lipid complexes on baking properties. –Part II.) *Sütőipar, 34,* 140–148.

KÁRPÁTI, M., BÉKÉS, F., LÁSZTITY, R., ÖRSI, F., SMIED, I. & MOSONYI Á. (1988): Wheat protein-lipid interactions and their role in baking technology. *8. Int. Cereal Bread Congr.,* Lausanne.

LARSEN, N. G., BARUCH, D. W. & NIEGEL, A. B. (1985): Lipid quality factors in breeding of New Zealand wheats. *Proc. 31. Austr. Cer. Chem. Conf.*, Sydney, pp. 239–244.

LARSEN, N. G., BARUCH, D. W. & NIEGEL, A. B. (1986): Lipid quality factors in breeding of New Zealand wheats. *Proc. Cer. Chem. Conf.* Wellington, pp. 124–130.

LÁSZTITY, R., BÉKÉS, F., KÁRPÁTI, M. & SMIED, I. (1987): A búzalipidek valamint a fehérje-lipid komplexek hatása a sütőipari minőségre. I. (The effects of wheat lipids and their protein-lipid complexes on baking properties. –Part I.) *Sütőipar, 34,* 64–67.

LÁSZTITY, R., BÉKÉS, F., ÖRSI, F., SMIED, I. & KÁRPÁTI, M. (1988): Protein-lipid and protein-carbohydrate interactions in the gluten complex. – in: LÁSZTITY, R. & BÉKÉS F. (Eds.) *Proc. 3. Int. Workshop of Gluten Proteins.* World Publ. Comp., Singapore, pp. 343–363.

LELLEY, J. (1973): Einzelpflanzenauslese und Mechanisierung in der Weizenzüchtung. *Z. Pfl. Zücht., 69.*, 129–134.

LUKOW, O. M., BÉKÉS, F. & BUSHUK, W. (1985): Influence of germination of wheat quality. – Part III. Modification of flour lipids. *Cereal Chem., 62,* 419–422.

MOOR, J. (1975): *A technológiai irányítás és preventív minőség ellenőrzés módszereinek korszerűsítése, sütőipari minőség védelemmel és szabványosítással kapcsolatos alapozó kutatások.* (Basic studies on the developments in technological regulation, preventive quality and standardization in baking industry.) Research Bulletin., Research Institute of Milling and Baking Industry, Budapest.

MORRISON, W. R. (1983): Acyl lipids in cereals. – in: BARNES, J. P. (Ed.) *Lipids in cereal technology.* Academic Press New York, pp. 11–32.

MORRISON, W. (1987): Control of some factors in cereal raw materials affecting the quality of processed foods. *Proc. 8. Natl. Convention Royal Austr. Chem. Inst.*, pp. 8–27.

MORRISON, W. R., TAN, S. L. & HARGIN, K. D. (1980): Methods for the quantitative analysis of lipids in cereal grains and similar tissues. *J. Sci. Fd Agric., 31,* 329–340.

PALLAGI, E., ALMÁSSY, K., ÖRSI, F. & MATUZ, J. (1985): Szedimentációs tesztek alkalmazásának vizsgálata búzalisztek minősítésére. (Use of sedimentation tests in the determination of wheat flour quality.) *Élelm. Ipar., 39,* 475–478.

PANOZZO, J., OBRIEN, L. & MACRITCHIE, F. (1987): Flour lipids and baking quality. *Proc. 33. Austr. Cer. Chem. Conf.* Parkville, Australia, pp. 1041–1042.

PANOZZO, J., OBRIEN, L., MACRITCHIE, F. & BÉKÉS, F. (1988): Prediction of breadmaking quality from flour lipid compositions. *Proc. 9. Conf. Austr. Plant. Breeding.*, Wagga-Wagga, pp. 341–343.

POMERANZ, Y. (1980): Interaction between lipids and gluten proteins. *Anns Technol. agric., 29,* 385–397.

SIMON-SARKADI P., & BÉKÉS, F. (1987): Personal communication.

VUK, M. (1929): A magyar búzaliszt összetétele. (The chemical composition of Hungarian wheat flour). Kilián, Budapest.

Acta Alimentaria, Vol. 19 (3), pp. 261—271 (1990)

AN ABSORPTION WEIGHING METHOD FOR DETERMINING THE DEGREE OF ENZYMATIC MACERATION IN FRUIT AND VEGETABLES

S. S. TANTCHEV[a], Y. MÄLKKI, E. PESSA, A. KINNUNEN and M. MOKKILA

Technical Research Centre of Finland, Food Research Laboratory,
SF-02150 Espoo. Finland

(Received: 6 July 1989; accepted: 6 November 1989)

A simple method to measure the degree of enzymatic maceration in fruits and vegetables has been developed. This method is based on the absorption of the liquefied fruit and vegetable samples into filter paper discs. After a sufficient diffusion time the insoluble residue is quantitatively collected from the filter paper and weighed. The applicability of the method was tested with apples, apricots, plums, green peppers, cucumbers and carrots. Factors which have an influence on the results, especially the temperature of the treatment, are demonstrated, as well as the connection between the maceration degree and the rheological properties of the samples. The method is also applicable under plant conditions, since no special devices and qualifications are required.

Keywords: enzymatic maceration, fruit and vegetable processing, rheological properties

Enzymatic maceration of the parenchymal tissue of fruit and vegetables is mainly applied for increasing the juice yield by pressing, as well as for improving the stability of turbid juices and nectars during storage. This is due to the enzymatic disintegration of the intercellular lamelly of parenchymal tissue, as well as to the breakdown of cell membranes. Small-sized pieces of parenchymal tissue, separate cells or groups of cells, or cell-wall fragments are obtained. Enzymatic disintegration of some plant polymers (mainly starch, cellulose and pectic substances) to smaller sized polymers, oligomers and monomers is achieved. This process is also called the liquefaction of fruit and vegetables. Its nature is described in detail by a number of authors (PILNIK et al., 1975; CHESSON, 1980; PILNIK & ROMBOUTS, 1981; VORAGEN & PILNIK, 1981; PILNIK, 1982; JANDA & DÖRREICH, 1984). Some particularities about the maceration mechanism still remain obscure and for that reason studies are underway.

One problem regarding this enzymatic process is the absence of an appropriate method for determining the degree of maceration. Methods presented so far have been based on the weight loss fruit or vegetable pieces to be macerated (ZETALAKI-HORVÁTH, 1974), on sieving (ZETELAKI-HORVÁTH &

[a] Permanent address:
Higher Institute for Food and Flavouring Industries, Plovdiv, Bulgaria

GÁTAI, 1977; SREENATH et al., 1984) or centrifugal separation (ZETELAKI-HORVÁTH & URBÁNYI, 1978) of the fragments, on the determination of viscosity of the macerate (STRUEBI et al., 1978; DONGOWSKI & BOCK, 1980; VORAGEN et al., 1980; JANDA & DÖRREICH, 1984), on determining the volume (SREENATH & RADOLA, 1966) or size distribution (ZETALAKI-HORVÁTH, 1980) of the precipitate of the macerate, on determining the dry matter (ANASTASAKIS et al., 1987) or reducing sugars (SREENATH et al., 1984; CALL et al., 1985) of the macerate, or on microscopic studies (SREENATH & RADOLA, 1966; VORAGEN et al., 1980; JANDA & DÖRREICH, 1984; SREENATH et al., 1984; ANASTASAKIS et al., 1987). None of these methods is easily applicable under processing plant conditions.

In the present study, the objective was to develop a simple and rapid method for evaluating the degree of maceration of the parenchymal tissues of fruit and vegetables under the influence of enzyme preparations having a macerating effect.

1. Materials and methods

1.1. Raw materials

Tests were conducted on commercial apples, apricots, plums, green pepper, cucumbers and carrots without identifying their variety or origin. Carrots had an average weight of about 120 g. After peeling with an abrasive corund kitchen peeler, carrots were cut lengthwise in four equal pieces and grated in strips about 2 mm thick. Strips were spread in a rectangular layer about 10 mm in height. An additional lengthwise cutting of the strips was achieved using a sharp kitchen knife by transverse perpendicular cuts at a distance of 1–1.5 cm on the layer. The fine fraction was separated on a sieve of 2×2 mm holes. The large fraction was spread on a thin layer between sheets of filter paper for separating the juice from the surface of the strips.

Apricots and plums were pitted, apples were cleaned free of seed chambers, and green peppers were separated from seed and placenta while cucumbers were peeled and cleaned of seeds. Using a surgical knife or razor, parenchymal tissue pieces having approximative dimensions of 2×2 mm and a length corresponding to the thickness of the parenchymal tissue of the respective fruit were formed. Pieces were then superficially dried with filter paper.

In order to ensure a homogeneous average sample, the fruit and vegetabe pieces thus prepared were well stirred prior to drying on a filter paper.

1.2. Enzymes

Enzyme preparations, Pectinex 3×1 (liquid) and Ultrazym M10 (powder) both by Novo A/S, Copenhagen, were used. Their activity prior to tests was not determined as it was not required by the objective of the studies. Enzymes

were dissolved in 0.1 mol l^{-1} buffer at pH = 4.0 $\pm$ 0.1, prepared from citric acid, hydrochloric acid and sodium hydroxide. The pH value of maceration was selected depending on the pH optimum of each enzyme. Ten concentrations of each enzyme were prepared. Using preliminary tentative tests, the concentrations enabling the achievement of distinct differences in the degree of maceration, according to the raw material and its degree of ripeness, were selected.

1.3. Maceration

Depending on the kind and the concentration of the enzyme and its activity, as well as on the origin of the parenchymal tissue, the enzyme treatment was conducted at room or elevated temperatures, using a shaking thermostat. The shaking rate was about 60 times per min. Due to occasional precipitation despite the shaking, a thorough stirring with a glass rod at intervals of 5 to 15 min was found to be necessary.

Samples of 1.0 g each (G_1) being prepared for maceration were put in glass test-tubes of volume about 20 cm^3. Five cm^3 of buffered enzyme solution was added. Samples were thoroughly stirred with a glass rod without exerting pressure on the pieces, and reporting the time of enzyme treatment started.

After the predetermined time of enzyme treatment, samples treated in the water bath were cooled without inactivating the enzyme, using running water, and the degree of maceration was immediately established. If sampling after enzyme treatment was delayed, or a greater accuracy was desired, the enzyme activity was reduced or the enzyme almost inactivated by adding a fixed amount (1–3 cm^3) of 30% citric acid solution to samples, in order to decrease the pH to about 2. Inactivation should not be achieved by thermal treatment. It was experimentally found that a sample of raw carrot pieces released about 15% of juice upon heating, and in the case of apples this percentage was even more important. A short-time storing of samples at 0 °C before further treatment was found possible.

1.4. Determination of the degree of maceration

The outlines of the method were as follows. The macerated mixture was drained on a wire mesh, and the solids transferred on a filter paper to absorb the liquid fraction. The remaining solid fraction consisting of tissue fragments, cells and cell fragments, was weighed (G_2). The degree of enzymatic maceration (Me) was determined according to the equation

$$\frac{G_1 - G_2}{G_1} \; 100 = \text{Me} \; (\%)$$

where G_1 is the weight of the sample.

The practical performance was as follows. Four discs of filter paper were placed in a Petri-dish of 9.0 cm diameter. Discs should be attached to one another after being put in the Petri dish without allowing contact with the walls nor to fold. This ensures a free moisture exchange between the respective paper discs. A plastic ring of 20 mm internal diameter and 3 mm in height was placed in the centre of the discs.

After enzyme treatment the content of the test-tube was transferred onto a metal net of 0.5×0.5 mm mesh, placed at an angle of about 30° on a beaker of about 100 mm in diameter. After decanting the samples and allowing them to drain for 5 min, the tissue particles were thoroughly collected using a putty-knife, without pressing, and transferred onto the filter paper surface confined by the plastic ring, and carefully spread in a uniform layer without pressing. The Petri-dish was closed and the sample was allowed to stand at room temperature for 3 h to ensure the absorption of the liquid fraction on the filter paper. The residue on the filter paper was then collected with a putty-knife without tearing the paper and weighed. G_2 was thus determined.

When developing the method, the influence of the filter paper, the number of discs and the time of standing on the results was examined. Whatman papers Nos. 1, 2, 3, 4, 40, 41 and 42 were tested.

The reproducibility of the method was tested using several concentrations of Pectinex 3×1 and Ultrazym M10 enzymes, 0.5 cm^3 of the enzyme solution being added directly on 5 g of grated carrots. Maceration was conducted by periodic stirring of samples at 40 °C. After the enzymatic treatment and cooling to 0 °C, each macerated sample was subjected to five parallel determinations taking 1.0 g of the macerate for draining and drying as suggested above. An important prediction was to carry out a thorough and intensive stirring of the sample using the putty-knife, and to perform sampling immediately after stirring was stopped. This ensured a normal liquid–solid fraction ratio of the sample.

1.5. Rheological measurements

Three samples of grated carrots, 800 g each, were treated with the enzyme preparation Pectinex 3×1 of 700 mg per kg concentration. The enzyme was directly added to the carrots and the samples were continuously stirred. After 60, 90 and 120 min, respectively, the enzyme was inactivated by heating the samples to about 85 °C in a microwave oven operating at a frequency of 2450 Hz, the time of heating being about 5 min. Subsequently, the samples were cooled and homogenized in a colloidal mill with a distance of 0.5 mm between the rolls. The paste-like sample obtained was subjected to rheological measurements using the Bohlin VOR Rheometer apparatus. The yield stress and strain sweep were determined at 25 °C. The yield τ_0 is defined as the shear

stress that has to be overcome to cause shear flow. The yield stress was obtained by extrapolation of the viscosity curve as a function of shear stress. Shear rates from 0.58 to 4.6 s^{-1} were used. The storage or elastic modulus G' was determined as a function of strain in the strain amplitude range of 0.01 to 20% and frequency of 0.2 Hz. The degree of maceration of the same samples prior to grinding was determined by the absorption-weighing method suggested.

2. Results

The results obtained from the studies are presented in Tables 1–3 and Figs. 1–4.

2.1. Practical performance

The most appropriate filter papers were found to be Whatman Nos. 1, 2, 4 and 41. The main requirement is that the number of discs is sufficient to ensure that the periphery should not be wetted from the liquid fraction absorbed during the three-hours test. Straining of the samples on the net should

Table 1

Reproducibility of the straining and drying step.
Carrot pieces treated with different concentrations of two enzymes

Enzyme	Conditions of maceration	Concentration (mg per kg)	Number of samples	G_2	
				Mean value (g)	Standard deviation
Ultrazym M10	t = 40 °C	25	5	1.036	0.048
	T = 80 min	50	5	1.016	0.018
	G_1 = 1.5 g	100	5	0.992	0.033
		150	5	0.89	0.057
		200	5	0.856	0.024
		300	5	0.74	0.017
Ultrazym M10	t = 40 °C	25	3	0.58	0.021
	T = 100 min	50	4	0.58	0.0171
	G_1 = 1.0 g	100	4	0.57	0.0082
		150	4	0.53	0.025
		200	5	0.44	0.023
		300	5	0.36	0.0217
Pectinex 3×1	t = 40 °C	75	4	0.35	0.0173
	T = 110 min	150	4	0.21	0.025
	G_1 = 1.0 g	300	4	0.12	0.0299
		450	5	0.14	0.0286
		600	5	0.09	0.0182
		900	5	0.13	0.02

be as complete as possible, otherwise the lower layer of paper might be abundantly wetted and attaches itself to the Petri-dish surface, which would diminish the uniformity of the absorption process. In order to eliminate possible attachment, a disc of plastic net with holes of about 1×1 mm and 8.0 cm in diameter should be put at the bottom of the Petri-dish.

In general every kind of filter paper may be used. A necessary condition is that the quantity of paper should ensure the absorption of the entire liquid fraction. When the sample has a low content of pectic substances, the duration of standing may be shorter (90–150 min). After the elapse of the period adopted, the sample should not be very wet, i.e. the absorption should be simply completed without permitting its drying.

2.2. *Reproducibility and extent of maceration*

According to the results of Table 1, the reproducibility of the draining and drying steps, when calculated as the degree of maceration (Me), is in general between 1 and 3%.

Data in Table 1 obtained for the maceration with Pectinex 3×1 show that the maceration of carrots was almost completed at 40 °C within 110 min

Table 2

Effect of the enzyme and its concentration on the maceration of carrot pieces at 40 °C

Enzyme	Treatment time (min)	Concentration (mg per kg)	Maceration (%)
Pectinex 3×1	120	75	52
		150	58
		300	67
		450	72
		600	75
		750	76
		900	78
		1000	77
		1200	76
		1300	79
Ultrazym M10	300	25	43
		50	48
		100	75
		200	77
		300	79
		400	79
		500	80
		600	81
		700	82
		800	90

when the enzyme concentration was 300 mg per kg, and adding the dosage of enzyme did not increase the maceration for the carrots used, although it evidently accelerated it. In the case of Ultrazym M10, the maceration was not completed after 100 min even at a concentration of 300 mg per kg.

When the enzymatic step was performed, the carrot pieces being added to the enzyme in the buffer solution, as described under Para. 1.3., results shown in Table 2 were obtained.

When comparing the results of Tables 1 and 2 it can be observed that in the case of Pectinex 3×1, a higher degree of maceration was achieved with a smaller amount of enzyme. This might be due to partial inhibition of the enzyme by citric acid. A comparable effect cannot be observed with Ultrazym M10, possibly due to the longer incubation time.

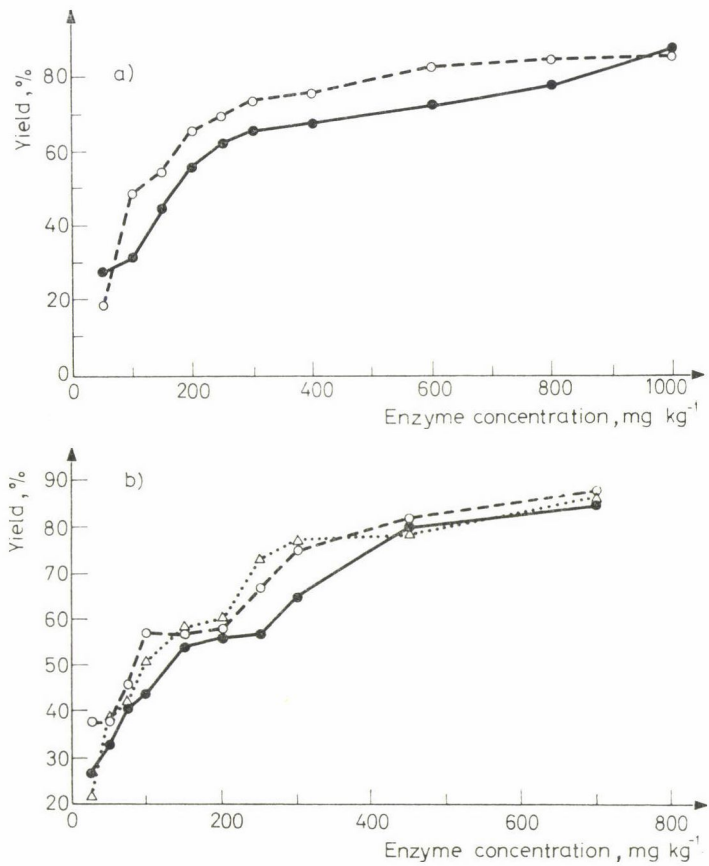


Fig. 1. Maceration of carrots with Ultrazym M 10. Influence of enzyme concentration and temperature on maceration yield
a): —●— 40 °C, 45 min; ---o--- 30 °C, 120 min;
b): —●— 20 °C, 170 min; ---o--- 40 °C, 40 min;
···△···· 30 °C, 80 min

The effects of time and temperature on the maceration of carrots are presented in Figs. 1 and 2. Temperature coefficients (Q_{10}) for the maceration reaction can be calculated from the maceration times, which give the same degree of maceration. The coefficients for both of the enzymes varied from 2.0 to 2.7.

For other materials, maceration tests were performed with Pectinex 3×1 only, and at room temperature (about 25 °C). Results are presented in Table 3.

With the exception of the lowest enzyme concentration, the results indicate a good dose and time response, which can be interpreted as an indication of the reliability of the method as applied to these materials.

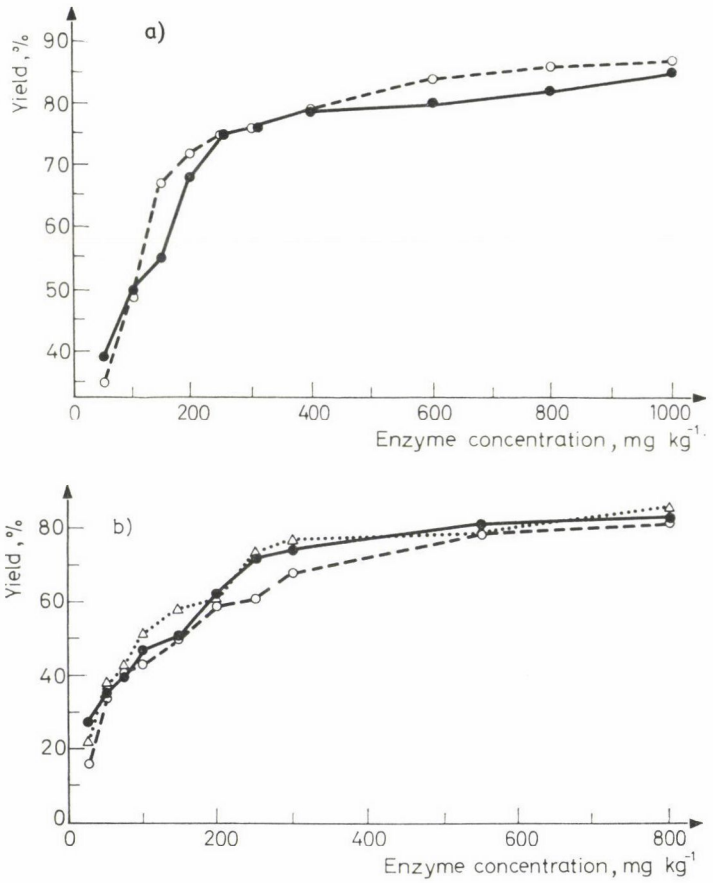


Fig. 2. Maceration of carrots with Pectinex 3×1. Influence of enzyme concentration and temperature on maceration yield
a): —●— 40 °C, 45 min; ---○--- 30 °C, 120 min;
b): —●— 40 °C, 40 min; ---○--- 30 °C, 80 min;
···△··· 20 °C, 170 min

Table 3

Effect of the kind of raw material, the enzyme concentration and the treatment time on the degree of maceration
(Enzyme: Pectinex 3×1; room temperature)

Raw material and treatment ttme (min)	Enzyme concentration (mg per kg)									
	50	100	150	200	250	350	450	650	850	1000
	Degree of maceration (%)									
Apricots — 30	61	65	69	76	71	73	76	80	—	83
Apricots — 15	49	48	50	54	57	64	68	77	75	78
Plums — 15	46	48	49	—	55	57	59	61	63	64
Green pepper — 180	28	27	34	36	39	42	51	51	—	53
Green pepper — 240	43	44	44	54	54	61	63	71	72	73
Green pepper — 300	52	51	53	64	63	64	72	79	81	82
Cucumbers — 210	65	66	69	78	78	85	88	89	92	95

2.3. Rheological studies

Results of the rheological studies (Figs. 3 and 4) indicate the correlation between the extent of maceration and the breakdown of the polymeric material of the plant tissue during maceration. The highest values of viscosity, yield value and storage modulus were obtained with the sample having a degree of maceration of 63%, the rheological values decreasing again when the maceration was continued, as expected. The rheological measurements indicate, that

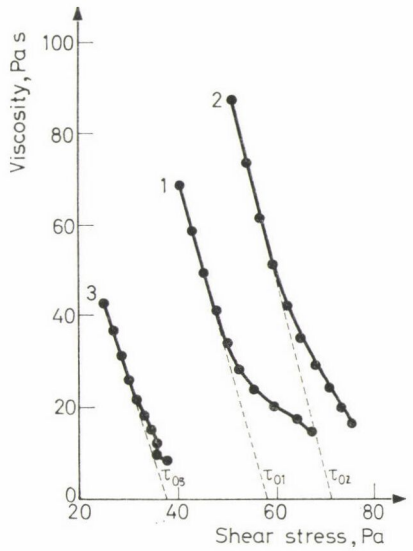


Fig. 3. Flow functions $\eta(\tau)$ and yield stresses τ_0 for carrot purées, macerated for 1 : 60 min., 2 : 90 min., 3 : 120 min. Degrees of maceration 51, 63, and 78%, respectively

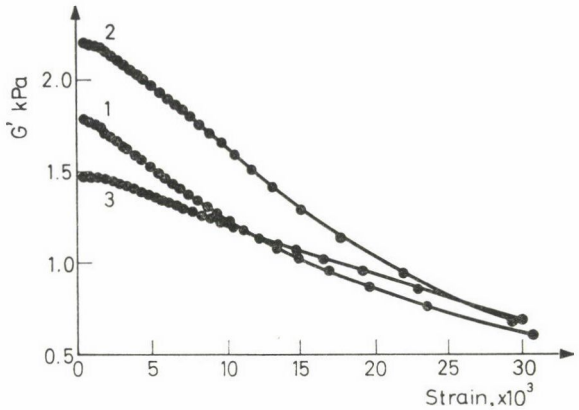


Fig. 4. Strain sweep curves for carrot purées as in Fig. 3

the method presented only shows the extent of maceration of the tissue, independently of how far the further breakdown is advancing. A more extensive study comprising several vegetable materials and various pretreatments is needed for defining the optimal degree of maceration.

3. Conclusions

The observed dose, temperature and time responses can be regarded as qualitative indications of the reliability of the method suggested. A few deviations in the degree of maceration from the expectations could be explained by the difference in the biochemical and morphological structure of the tissues of the samples for which these deviations were reported. It is evident that special attention should be paid to the preparation of the average sample. The small differences reported in some cases are due to slight differences in the enzyme concentrations. However, these peculiarities substantiate the authenticity and the sensitivity of the method suggested.

As compared to the methods suggested for the same purpose previously, the method developed is evidently more easily applicable under processing plant conditions, since no special laboratory devices except for a balance are required, and the performance can be easily learned. It also can give quantitative values for quality control and for comparing the different raw materials and enzymes.

*

The author Stoyan Stoychev TANTCHEV thanks the Ministry of Education of Finland for the granted scholarship and to the management and personnel of the Food Research Laboratory for the comprehensive assistance and collaboration in carrying out the present studies.

Literature

ANASTASAKIS, M., LINDAMOOD, J. B., CHISM, G. W. & HANSEN, P. M. T. (1987): Enzymatic hydrolysis of carrot for extraction of cloud-stable juice. *Fd Hydrocolloids, 1,* 247–261.

CALL, H. P., WALTER, J. & EMELS, C. C. (1985): Maceration activity of an endo-polygalacturonase from *Candida macedoniensis. J. Fd Biochem., 9,* 325–348.

CHESSON, A. (1980): A review: Maceration in relation to the post-harvest handling and processing of plant material. *J. appl. Bact., 48,* 1–45.

DONGOWSKI, G. & BOCK, W. (1980): Eine Prüfmethode zur Ermittlung der mazerierenden und depektinisierenden Eigenschaften von pektolytischen Enzympräparaten. *IFJU-Reports, 16,* 275–290.

JANDA, W. & DÖRREICH, K. (1984): Optimized apple mash treatment. *IFJU-Reports, 18,* 207–217.

PILNIK, W. (1982): Enzymes in the beverage industry. –in: DUPUY, P. (Ed.) *Proceedings of the International Symposium on the Use of Enzymes in Food Technology.* Technique et documentation Lavoisier, Paris, pp. 425–450.

PILNIK, W. & ROMBOUTS, F. M. (1981): Pectic enzymes. –in: BIRCH, G. G., BLAKEBROUGH, N. & PARKER, K. J.: *Enzymes and food processing.* Elsevier, London, pp. 105–128.

PILNIK, W., VORAGEN, A. G. J. & DE VOS, L. (1975): Enzymatische Verflüssigung von Obst und Gemüse. *Flüss. Obst, 42,* 448–451.

SREENATH, H. K., FREY, M. D., RADOLA, B. J. & SCHERZ, H. (1984): Degradation of washed carrot preparation by cellulases and pectinases. *Biotechnol. Bioeng., 26,* 788–796.

SREENATH, H. K. & RADOLA, B. J. (1966): The effect of removing cellulases from a commercial pectinase on maceration and liquefaction of carrots. *J. Biotechnol., 4,* 269–282.

STRUEBI, P., ESCHER, F. E. & NEUKOM, H. (1978): Use of macerating pectic enzyme in apple nectar processing. *J. Fd Sci., 43,* 260–263.

VORAGEN, F. G. J., KRIST, R., HEUTINK, R. & PILNIK, W. (1980): Apple cell wall digestion by polysaccharide degrading enzymes. –in: LINKO, P. & LARINKARI, J. (Eds.) *Food process engineering,* Vol. 2. *Enzyme engineering in food processing.* Applied Science Publishers, London, pp. 264–275.

VORAGEN, A. G. J. & PILNIK, W. (1981): Spezifische Enzymeinwirkungen bei der Verflüssigung von Apfelzellwandpräparaten. *Flüss. Obst, 48,* 261–264.

ZETELAKI-HORVÁTH, K. (1974): Methods for measuring macerase activity.—Part II. Determination on macerase activity from the weight loss. *Acta Alimentaria, 3,* 293–308.

ZETELAKI-HORVÁTH, K. (1980): Disintegration of vegetable tissues as a function of polygalacturonase concentration and incubation period. *Acta Alimentaria, 9,* 367–382.

ZETELAKI-HORVÁTH, K. & GÁTAI, K. (1977): Disintegration of vegetable tissues by endo-polygalacturonase. *Acta Alimentaria, 6,* 227–240.

ZETELAKI-HORVÁTH, K. & URBÁNYI, GY. (1978): Determination of particle size of vegetable tissue by a sedimentation technique after enzymatic disintegration. *Acta Alimentaria, 7,* 69–78.

Acta Alimentaria, Vol. 19 (3), pp. 273—279 (1990)

ANALYTICAL STUDIES INTO RADIATION-INDUCED STARCH DAMAGE IN BLACK AND WHITE PEPPERS

J. FARKAS, M. M. SHARIF and S. BARABÁSSY

Institute of Preservation and Livestock Products Technology,
University of Horticulture and Food Industry,
1118 Budapest, Ménesi út 43–45. Hungary

(Received: 26 July 1989; 12 December 1989)

In relation to efforts for developing methods of detection of radiation treatment, ground black pepper samples equilibrated to water activity levels of 0.25, 0.50 and 0.75 a_w, resp., have been irradiated with gamma radiation doses of 0, 4, 8, 16 or 32 kGy and their damaged starch content, reducing sugar content and alcohol induced turbidity of their aqueous extracts, were investigated. Gelatinization thermograms of aqueous suspensions of the same samples plus those of ground white pepper were obtained by differential scanning calorimetry. The colorimetric method and the alcohol-induced turbidity showed statistically significant increases of starch damage at 4 kGy or higher dose levels. However, all investigated analytical indices of starch radio-depolymerization have been changed less dramatically by irradiation than the apparent viscosity of the gelatinized suspensions of spices which has been reported previously.

Keywords: irradiation of peppers, detection of radiation treatment, starch damage

In accordance with the findings of MOHR and WICHMANN (1985) and HEIDE and co-workers (1987, 1988), we have reported dramatic decreases of dispersion viscosity of heat gelatinized suspensions of several irradiated spices of considerable starch content as compared to that of unirradiated samples (FARKAS et al., 1990 a, b), and this may provide a relatively simple diagnostic technique for the detection of irradiation treatment of such spices.

Because the above effect seemed to be related to the radio-depolymerization of starch in the irradiated spices, additional analytical techniques have been tried to investigate starch damage in black and white peppers. The results will be reported as follows.

1. Materials and methods

1.1. Spices

Whole black and white pepper samples were received from a spice trade company, Budapest, and were finely ground for irradiation and analysis.

In order to study the effect of moisture conditions during irradiation, ground black pepper samples were equilibrated to 25%, 50%, and 75% relative humidities (a_w 0.25, 0.50 and 0.75), respectively, over sulphuric acid

solution at ambient temperature before irradiation and analysis. White pepper samples were investigated at their original a_w level of approx. 0.5.

1.2. Irradiation

Irradiation was carried out in a self-shielded Co-60 irradiator type RH-gamma-30 at the Central Food Research Institute, Budapest, under aerobic conditions and at ambient temperature. The dose rate of the treatment varied between 7.5 to 7.0 kGy h^{-1} during the period of the experiments.

1.3. Starch damage

Damaged starch was determined colorimetrically by the method proposed by WILLIAMS and FEGOL (1969). The test is based on the colour developed on addition of iodine to an extract made by treating samples with a solvent of sodium sulphate solution containing 15% formamide, and 0.2% sulphosalicylic acid. Results are recorded as absorbance at 555 nm.

1.4. Reducing sugar content

Reducing sugar content was estimated by the A.O.A.C. method for wheat flour (A.O.A.C., 1960).

1.5. Alcohol-induced turbidity of the aqueous extract

Following the observation on irradiated wheat flour reported by DESCHREIDER (1969), alcohol-induced turbidity in hot water extract of pepper samples, was studied. Five g of ground spice were extracted at 60 °C for 30 min with 45 cm^3 distilled water. After cooling, the suspension was centrifuged at 10 000 r.p.m. for 20 min, and the supernatant was filtered over a folded filter MN 615 1/4. For the turbidity test, 2 cm^3 of filtrate were mixed with 4 cm^3 of 96% ethyl alcohol, and the absorbancy was measured at 540 nm.

1.6. Differential scanning calorimetry

Gelatinization thermograms of aqueous suspensions of ground black pepper were produced by differential scanning calorimetry (DSC) in a DuPont 910 DSC-module. The instrument was calibrated with an indium standard. Ground pepper of 1.5–3 mg was placed in aluminium sample pans with measured amounts of distilled water to obtain a slurry of approx. 85% moisture content, hermetically sealed, and allowed to equilibrate 2–3 h prior to analysis. A pan filled with distilled water was used as a reference. Samples were heated at a rate of 10 °C per min from 25 °C to 115 °C. Thermograms were recorded with a Riken Danshi X-Y plotter. The onset temperature of the endotherm (T_o) and the peak temperature (T_p) were determined from the thermogram.

2. Results and discussion

2.1. Damaged starch in black pepper

Colorimetric reaction for estimating damaged starch was performed with black pepper samples. The absorbance of extracts at 555 nm after the reaction with the iodine reagent of WILLIAMS and FEGOL (1969) was used as measure of starch damage. Results are summarized in Table 1.

Table 1

Damaged starch in ground black pepper estimated colorimetrically by the method of WILLIAMS *and* FEGOL (1969)

Radiation dose (kGy)	Absorbance at 555 nm		
	$a_w = 0.75$	$a_w = 0.50$	$a_w = 0.25$
0	0.37[+]a	0.40 ab	0.38 a
4	0.54 bc	0.55 bc	0.55 bc
8	0.66 cd	0.67 cd	0.70 d
16	0.89 e	1.01 ef	1.16 f
32	1.53 g	1.85 h	1.95 h

[+] Means of duplicate measurements
Values marked by the same letter are not significantly different ($P \leq 0.05$)

As shown by analysis of variance, radiation treatment increased the damaged starch content. Up to 8 kGy dose, the water activity of samples did not influence significantly the starch damage values. At higher dose levels, the radiation-induced starch-damage increased with decreasing water activity of the irradiated sample.

2.2. Reducing sugar content in black pepper

Reducing sugar contents of black pepper samples as a function of radiation dose and water activity are shown in Table 2. Measurements were performed within one month after irradiation.

Table 2 shows that a significant increase of the reducing sugar content was observed in samples of 0.75 a_w at 8 kGy or higher doses. Decreased starch content and increased sugar content were reported by HEWAMANNA and BOTEJU (1985) in irradiated black pepper. It is known from radiation chemistry of pure starches that reducing power increased as the irradiation dose increased and probability of cleavage of glucosidic bonds depended upon the water content of starch (RAFFI et al., 1981). Similarly, Egyptian authors found that total soluble sugars and reducing sugars in corn grains increased

Table 2

Reducing sugar content expressed as maltose concentration in black pepper

Radiation dose (kGy)	Reducing sugar content (mg per 10 g)		
	$a_w = 0.75$	$a_w = 0.50$	$a_w = 0.25$
0	75+a	77 ab	85 abc
4	88 abc	79 ab	84 abc
8	96 cd	83 abc	81 abc
16	97 cd	89 abc	85 abc
32	107 d	91 bcd	98 cd

+ Means of duplicate measurements made within one month after irradiation

Values marked by the same letter are not significantly different

with increasing dose level of gamma irradiation (EL SAADANY et al., 1976, ROUSHDI et al., 1983).

2.3. Alcohol-induced turbidity of the aqueous extract of black pepper and white pepper

Alcohol-induced turbidities in hot-water extract of black pepper and white pepper samples are shown in Table 3.

Measurements were performed approx. two months after irradiation. Due to the coloured compounds extracted from the skin particles of black pepper, its extract showed higher absorbancy than that of white pepper. While white pepper of regular a_w (around 0.5) showed significant increase of turbidity already at 4 kGy dose level, the increase of turbidity was less pro-

Table 3

Alcohol-induced turbidity of aqueous extracts
(Absorbancy of aqueous extracts at 540 nm after mixing with 96% ethylalcohol in 1 : 2 w/v ratio)

Radiation dose (kGy)	Black pepper			White pepper
	$a_w = 0.75$	$a_w = 0.50$	$a_w = 0.25$	
0	0.64+a	0.87 bc	0.88 bc	0.14 a
4	0.81 ab	0.93 bcd	0.85 bc	0.39 b
8	0.87 bc	0.94 bcd	0.98 cd	0.43 c
16	0.94 bcd	0.83 ab	1.05 d	0.49 d
32	0.93 bcd	0.94 bcd	1.45 e	0.75 e

+ Means of duplicate measurements

Values marked by the same letter are not significantly different. Test comparisons are only within the same spice

nounced in black pepper. The a_w-effect noticeable in black pepper data at higher doses is related probably to findings of RAFFI and co-workers (1981) namely that water has an inhibiting effect upon the formation of water-soluble radio-dextrins in starch.

2.4. DSC measurements

The DSC thermograms of aqueous pastes of ground pepper samples showed an endothermal peak characteristic of starch gelatinization. The onset and peak temperatures of the endotherms are summarized in Table 4 for both black pepper and white pepper samples, untreated or irradiated.

Table 4

Characteristic temperature values of DSC-endotherms of untreated and irradiated pepper suspensions of approx. 85% moisture content

Samples	Number of replicates	T_0 (°C)		T_0 (°C)	
		$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Black pepper					
0 kGy	6	81.3	0.6	84.8	0.7
4 kGy	6	80.8	1.1	84.9	0.8
8 kGy	3	81.6	0.8	84.8	1.2
16 kGy	3	80.7	0.8	84.7	0.2
32 kGy	7	79.4	1.5	83.7	0.6
White pepper					
0 kGy	4	76.5	1.1	80.3	0.9
8 kGy	5	76.1	1.2	80.6	0.8

As it can be seen, the endothermal transition occurred at a higher temperature in case of black pepper than in white pepper. Although on the basis of literature on pure starches (OROTSCHENKO & KOROTSCHENKO, 1961, WOOTON & BAMUNVARACHCHI, 1979) a decrease in gelatinization energy and a shift of the endothermal change to lower temperatures were expected, except a tendency for slightly lower characteristic temperatures of the 32 kGy-irradiated black pepper, the endothermal curves showed insignificant differences between untreated and irradiated samples. Due to the preliminary and qualitative character of these studies, enthalpies were not calculated, however, no apparent differences were noted in the size of the endothermal peaks.

Our observations are in agreement with those of HOFREITER and RUSSEL (1974) who found that the initial gelatinization temperature of irradiated corn starch did not significantly deviate from the initial gelatinization temperature of untreated starch as the radiation dose was increased up to 5 kGy.

3. Conclusions

The analytical techniques measuring damaged starch content colorimetrically, reducing sugar content and alcohol-induced turbidity of hot-water extracts, respectively, showed an increased starch damage in pepper samples as a function of radiation dose. However, these indices have been changed less dramatically by irradiation than the apparent viscosity of the gelatinized suspensions of spice (FARKAS et al., 1990a, b). The moisture content influenced partial radio-depolymarization of starch in the samples. According to our preliminary experiments, the DSC-technique measuring the energy and temperature characteristics of heat gelatinization of starches, can not rival the sensitivity of viscometric measurement, in detection of radiation induced changes. Therefore, testing samples of various origin to estimate the "natural" variation and the factors influencing identification of radiation-induced changes will be continued with viscometric techniques.

*

This work is part of the IAEA Research Agreement No. 5348/CF.
The assistance of Mr Zoltán MOLNÁR in DSC-measurements is gratefully acknowledged. The experiments have been sponsored by the Central Food Research Institute, Budapest.

Literature

A.O.A.C. (1960): *Official methods of analysis of the Association of Official Agricultural Chemists.* 9th ed. Association of Official Agricultural Chemists, Washington, D.C., USA, pp. 162–164.

DESCHREIDER, A. R. (1969): Turbidimetrie et colorimetrie des polysaccharides de la farine de blé. Application eventuelle aux produits irradiés. *Lebensm. Wiss. u. Technol., 2,* 90–93.

EL SAADANY, R. M. A., EL SAADANY, F. M. & FODA, Y. H. (1976): Degradation of corn starch under the influence of gamma irradiation. *Stärke, 28,* 208–221.

FARKAS, J., KONCZ, Á., & SHARIF, M. M., (1990a): Identification of irradiated dry ingredients on the basis of starch damage. — in: *Proc. of the 7th Int. Meet. on Radiation Processing, Noordwijkerhout. The Netherlands, 24–28 April 1989. Rad. Phys. Chem., 35,* 324–328.

FARKAS, J., SHARIF, M. M. & KONCZ, Á. (1990b): Detection of some irradiated spices on the basis of radiation induced damage of starch. *Rad. Phys. Chem.* (In press.)

HEIDE, L., MOHR, E., WICHMANN, G., ALBRICH, S. & BÖGL, K. W. (1987): *Viskositätsmessung — Ein Verfahren zur Identifizierung strahlenbehandelter Gewürze?* ISH-Heft 120, Institut für Strahlenhygiene des Bundesgesundheitsamtes, Neuherberg b/München, 71 pp.

HEIDE, L., MOHR, E., WICHMANN, G. & BÖGL, K. W. (1988): Are viscosity measurements a suitable method for the identification of irradiated spices? — in: BÖGL, K. W., REGULLA, D. F. & SUESS, M. J. (Eds) *Health impact, identification, and dosimetry of irradiated foods.* ISH-Heft 125, Institut für Strahlenhygiene des Bundesgesundheitsamtes, Neuherberg b/München, pp. 176–189.

HEWAMANNA, R. & BOTEJU, L. W. (1985): Microbiological and chemical studies on irradiated black pepper. *Int. J. appl. Rad. Isot., 36,* 989–990.

HOFREITER, B. T. & RUSSEL, C. R. (1974): Gamma-irradiated corn starches. Alkaline dispersions for surface-sizing paper. *Stärke, 26,* 18–23.

MOHR, E. & WICHMANN, G. (1985): Viskositätserniedrigungen als Indiz für eine Cobaltbestrahlung an Gewürzen? *Gordian, 85,* (5), 96.

OROTSCHENKO, W. F. & KOROTSCHENKO, K. A. (1961): Untersuchung der Abbau von Stärke unter der Einwirkung der Gamma-Strahlung, *Stärke, 5,* 183–184.

RAFFI, J., DAUBERTE, B., D'URBAL, M., POLLIN, C. & SAINT-LÉBE, L. (1981): Gamma radiolysis of starches derived from different foodstuffs.—Part IV. Study of radiodepolymerization. *Stärke, 33,* 301–306.

ROUSHDI, M., HARRAS, A., EL-MELIGI, A. & BASSIM, M. (1983): Effect of high doses of gamma rays on corn grains. — Part II. Influence on some physical and chemical properties of starch and its fraction. *Stärke, 35,* 15.

WILLIAMS, P. C. & FEGOL, K. S. W. (1969): Colorimetric determination of damaged starch in flour. *Cereal Chem., 46,* 56–62.

WOOTON, M. & BAMUNVARACHCHI, A. (1979): Application of differential scanning calorimetry to starch gelatinization. — Part I. Commercial native and modified starches. *Stärke, 31,* 201–208.

Acta Alimentaria, Vol. 19 (3), pp. 281–290 (1990)

EFFECT OF GAMMA IRRADIATION AT DOSES OF 5–15 kGy ON THE QUALITY PROPERTIES OF DURUM WHEAT SEMOLINA

S. A. TAHA

Department of Biochemistry, Faculty of Agriculture,
Zagazig University, Zagazig. Egypt

(Received: 15 September 1989; accepted: 15 December 1989)

Semolina purified from four durum wheat varieties were gamma-irradiated with 0, 5, 10 and 15 kGy, i.e. at dose levels expected to improve the colour of pasta products, suitable for reduction of microbial contamination and high above doses required for insect disinfestation. The semolinas were then assessed for colour indices, carotenoid content, oxidative enzyme activities, mixing properties, gluten strength and cooking quality. The irradiation resulted in significant losses in carotenoid content and oxidative enzyme activities. Significant increases in yellowness and decreases in brownness were also observed. Gamma irradiation slighty affected the mixing properties, greatly reduced the gluten strength and adversely affected firmness of cooked pasta as well as its tolerance to overcooking. Consequently, preservative gamma irradiation of durum wheat semolina should be limited to a maximum dose of 5 kGy and pasta prepared from irradiated-semolina should not be cooked longer than 15 minutes.

Keywords: durum wheat quality, pasta quality, gamma irradiation, colour indices, oxidative enzymes, cooking quality

Currently, a major problem in Egypt is that the domestic production of wheat does not satisfy the annual requirements of the Egyptian population. Therefore, Egypt imports about 75% of the bread wheat demand as grains or flour and 100% of durum wheat demand as high quality semolina. However, these materials may become contaminated with various insects and microorganisms (INGRAM & FARKAS, 1977), depending on their moisture content and storage conditions, particularly temperature and humidity (BECZNER et al,. 1983). Gamma irradiation of stored foods, including cereal grains and their products, as a method for control of both insect infestation and microbial contamination has been the subject of considerable research (URBAIN, 1986). It is generally agreed that the irradiation doses required to eliminate insects are quite low and usually do not exceed 0.5 kGy. The necessary doses for efficient control of microbial contamination are usually higher than 1 kGy (INGRAM & FARKAS, 1977). However, such doses have been shown to affect quality properties, chemical and nutritional components of radiation-treated cereals. Rheological properties and baking quality of bread wheat flour (EL-SAADANY, 1979); quality properties of soybeans (BECZNER et al., 1983) and rice (WOOTTON et al., 1988); carotenoid compounds isolated from paprika

(ZACHARIEV & KISS, 1982) and nutritional quality of cereal grains and legumes (HANIS et al., 1988; BADSHAH & KLOPFENSTEIN, 1989a, 1989b) were studied as affected by gamma irradiation. However, the effect of high dose gamma irradiation on the quality properties of durum wheat and pasta products has not been studied yet.

Thus, in the present study, I have irradiated purified semolinas from four durum wheat varieties with 5–15 kGy doses and have examined the effect of dose level on colour indices, carotene content, oxidative enzyme activities, gluten strength, mixing properties and cooking quality.

1. Materials and methods

1.1. Materials

Grain samples of four durum wheat varieties, three Hungarian (GK Basa, GK Minaret, SZD8) and one Egyptian (Stork-s) with various quality properties (TAHA & SÁGI, 1987a, b) were obtained from the Wheat Breeding Division, Cereal Research Institute, Szeged, Hungary. All the seed samples were produced at the same location in the same year, using standard agronomic practices.

1.2. Milling and purification

The grain samples were tempered to 14.5% moisture, milled in a modified ÉLGÉP laboratory mill and purified with a laboratory purifier, furnishing particle sizes of 200–400 μm.

1.3. Irradiation

Samples of purified semolina from each variety (about 500 g) were irradiated with doses of 5, 10 and 15 kGy from a ^{60}Co gamma cell at the Microbiology Division, Central Food Research Institute, Budapest, Hungary. Non-irradiated semolinas served as controls. Before use, all samples were stored in a refrigerator at 4 °C.

1.4. Colour indices

For the determination of semolina yellow index (YI) and brown index (BI), the modified method of ALAUSE and FEILLET (1970) as described by TAHA and SÁGI (1987a), was used.

1.5. Gluten quality

Gluten strength was assessed by the sodium dodecyl sulfate (SDS) sedimentation test according to AXFORD and co-workers (1978).

Mixing properties were determined by both the 10-gram-micromixograph (FINNEY & SHOGREN, 1972) and the 50-gram-farinograph (IRVINE et al., 1961) techniques, based upon the parameters dough development time (DDT), maximum consistency (MC), tolerance index (TI) and curve area (CA).

1.6. Cooking quality

Firmness of 10 discs (5 cm diameter) of dried pasta prepared from the semolina was determined after normal cooking for 15 min (5 discs) and after overcooking for 30 min (5 discs) by the aleurograph test as described by SCOTTI and co-workers, 1976).

1.7. Chemical analyses

1.7.1. Carotene content. Carotenoid pigments were extracted overnight from 8 g semolina with 40 cm^3 water-saturated n-butanol, filtered and the light absorption of the clear filtrate was measured at 440 μm according to AACC (1968).

1.7.2. Oxidative enzyme activities. Lipoxygenase (LPO), polyphenoloxidase (PPO) and peroxidase (PO) activities were determined spectrophotometrically using a Pye-Unicam recording spectrophotometer with a thermostated cell at 25 °C according to MCDONALD (1979) and SZÁNTHÓ and co-workers (1981) for LPO, HONOLD and STAHMANN (1968), KRUGER (1976) and LAMKIN and co-workers (1981) for PPO and HONOLD and STAHMANN (1968) and KOBREHEL and co-workers (1974) for PO.

1.8. Statistical analyses

The data presented here are the averages of three parallel determinations.

Results obtained at all 3 irradiation levels were pooled for statistical evaluation. Significant differences at 5% (SD 5%) and F test were calculated with a Commodore 64 personal computer according to SVÁB (1981).

2. Results and discussion

2.1. Colour and related factors

The colour, appearance and behaviour during cooking are the main criteria of pasta quality. The colour of pasta has two contrasting components: the yellow and the brown.

Table 1

Effect of gamma irradiation on carotenoid pigments, oxidative enzyme activities and colour indices of semolina milled from four durum wheat varieties

Variety and dose (kGy)	Carotenoid pigments		Oxidative enzyme activities						Colour indices	
			PO		PPO		LPO			
	content (mg per kg)[a]	loss (%)	(EU per 100 g)[a]	loss (%)	(EU per 0.1 g)[a]	loss (%)	(EU per 1.0 g)[a]	loss (%)	yellow	brown
GK Basa										
0.0	3.78		653		166		432		23.1	35.0
5.0	3.51	7.14	610	6.6	129	22.3	293	32.2	24.3	34.9
10.0	3.30	12.70	422	35.4	92	44.6	140	67.6	24.8	34.1
15.0	3.08	18.52	314	51.9	72	56.6	61	85.9	23.9	32.9
GK Minaret										
0.0	5.06		522		138		364		26.1	34.7
5.0	4.83	4.55	431	17.4	96	30.4	269	26.1	27.7	33.5
10.0	4.52	10.67	280	46.4	75	45.7	133	63.5	26.6	32.8
15.0	4.34	14.23	193	63.0	48	65.2	58	84.1	27.2	31.9
Stork-s										
0.0	5.03		710		152		411		27.5	33.2
5.0	4.41	12.33	523	26.3	101	33.6	272	33.8	28.3	32.3
10.0	4.34	13.72	381	46.3	87	42.8	176	57.2	28.3	31.9
15.0	3.41	32.21	232	67.3	53	65.1	50	87.8	27.5	30.9
SZD8										
0.0	5.28		444		93		448		28.7	31.2
5.0	5.07	3.98	380	14.4	52	44.1	253	43.5	31.0	30.6
10.0	4.68	11.36	245	45.3	36	61.3	109	75.7	29.6	28.7
15.0	4.25	19.51	151	66.0	21	77.4	42	90.6	28.7	28.2
SD 5% between varieties	0.31	5.60	58.74	6.40	10.50	7.70	39.89	6.90	0.81	0.85
SD 5% between treatments	0.31	5.60	58.74	6.40	10.50	7.70	39.89	6.90	0.81	0.85
F test for varieties	***	NS	***	*	***	*	NS	NS	***	***
F test for treatments	***	***	*****	***	***	***	***	***	*	***

* Significant at P = 95% probability level
*** Very highly significant at P = 99.9% probability level
NS: non-significant
[a]: on dry weight basis

The effects of gamma irradiation on carotenoid pigments, oxidative enzyme activities and colour indices of semolina milled from the four durum wheat varieties are shown in Table 1. Due to the irradiation, considerable losses of carotenoid pigments and all oxidative enzyme activities were observed. Carotenoid losses were significant at the high radiation doses (10 and 15 kGy), while decreases of oxidative enzyme activities were often significant at the

lowest dose (5 kGy). Among the oxidative enzymes, LPO showed a greater sensitivity to radiation than both PO and PPO. PO was least affected by irradiation. These relatively high decreases of oxidative enzyme activities upon radiation treatment might be due to the higher moisture content of semolina samples during irradiation. The moisture content ranged between 13.8 and 14.6% compared to those of wheat samples before milling which were in the range from 10.1 to 11.2%. The moisture content significantly influences the effect of radiation treatment. Data presented in Table 1 also revealed that significant increases of semolina yellow index (SYI) and highly significant decreases of semolina brown index (SBI) were caused by gamma irradiation, indicating that improvement of pasta colour can be obtained by irradiation of raw semolina. The best yellow colour was obtained often at the radiation dose of 5 kGy, while the brownness weakened as the radiation dose increased. Yellowness of pasta products have been established as a function of carotenoid pigment content of semolina and LPO activity (IRVINE & WINKLER, 1950; IRVINE & ANDERSON, 1953; MATSUO et al., 1970; WALSH et al., 1970; LAIGNELET, 1983; TAHA & SÁGI, 1987a). The destruction of carotenoids during pasta processing is due to a coupled oxidation reaction catalysed by the semolina-LPO (IRVINE & WINKLER, 1950; IRVINE & ANDERSON, 1953). Accordingly, the increases of SYI under the effect of irradiation in spite of the significant losses of carotenoid pigments (Table 1) can be explained by partial inactivation of the oxidative enzymes including LPO and indirect protection of carotenoids against destruction by LPO during processing. Irradiation with 5.0, 10.0 and 15.0 kGy decomposed 7, 12 and 21% of semolina carotene content and inactivated 34, 66 and 87% of LPO activity, respectively.

The brownness of pasta products as related to the semolina oxidative enzyme activities, particularly, to PO and PPO has been demonstrated (MENGER et al., 1969; KOBREHEL et al., 1972; 1974; KOBREHEL & GOUTIER, 1973; TAHA & SÁGI, 1987a). Therefore, the highly significant decreases of SBI resulting from gamma irradiation (Table 1) may, at least partially, be due to the inactivation of PO and PPO under the effect of radiation. Improvement of PO and PPO under the effect of radiation. Improvement of yellow colour can be a result of the decreased brownness, too.

2.2. Cooking quality and related factors

Strong mixing properties and high gluten strength together with high protein content of semolina have been established as the main bases of superior pasta cooking quality (MATVEFF, 1966; IRVINE, 1971; DEXTER & MATSUO, 1977, 1978; DEXTER et al., 1988; FEILLET, 1983; TAHA & SÁGI, 1986, 1987b).

Table 2 shows the effects of gamma irradiation upon mixing properties of semolina purified from the four varieties examined as determined by farino-

Table 2

Effect of gamma irradiation on mixing properties of semolina milled from four durum wheat varieties

Variety and dose (kGy)	Farinograph parameters						Mixograph parameters		
	DDT[a] (mm)	MC[b] (BU)	TI[c] (BU)	AUC[d] (cm²) up to MC[b]	AUC[d] (cm²) total	quality value	DDT[a] (mm)	MC[b] (MU)	AUC[d] (cm²)
GK Basa									
0.0	34.0	648	81	15.6	63.9	75.5	56.0	491	21.0
5.0	39.5	624	100	16.5	69.6	72.8	52.3	474	21.0
10.0	37.5	605	105	18.4	69.5	71.9	45.5	435	15.0
15.0	33.0	532	92	14.5	59.5	73.8	37.5	403	10.2
GK Minaret									
0.0	43.5	540	62	13.7	60.8	78.4	44.0	491	15.4
5.0	48.0	556	54	15.8	64.8	80.0	44.5	443	14.7
10.0	44.5	529	35	13.4	60.8	84.5	46.5	426	13.8
15.0	45.0	502	27	14.0	59.3	86.2	46.0	408	9.5
Stork-s									
0.0	61.0	521	33	18.2	77.0	84.7	55.5	495	22.0
5.0	45.0	483	30	12.1	55.5	85.2	55.0	480	18.4
10.0	46.5	478	35	14.1	56.7	84.0	56.5	458	18.4
15.0	43.5	470	41	12.0	53.6	82.9	54.5	526	15.5
SZD8									
0.0	45.0	548	43	14.6	63.4	82.5	56.0	535	22.4
5.0	41.0	529	57	13.2	59.6	79.7	55.0	496	21.1
10.0	40.0	518	49	12.6	58.4	81.1	54.3	472	18.5
15.0	40.0	489	27	13.5	57.5	86.2	56.0	430	14.2
SD 5% between varieties	7.31	28.71	19.3	2.95	9.38	4.01	6.86	15.25	2.50
SD 5% between treatments	7.31	28.71	19.3	2.95	9.38	4.01	6.86	15.25	2.50
F test for varieties	*	***	***	NS	NS	***	*	***	**
F test for treatments	NS	**	NS	NS	NS	NS	NS	***	***

* Significant at P = 95% probability level
** Highly significant at P = 99% probability level
*** Very highly significant at P = 99.9% probability level
NS: non significant
[a]: dough development time, [b]: maximum consistency, [c]: tolerance index, [d]: area under curve

graph and mixograph tests. As obvious in Table 2, the farinograph parameters of semolina did not change considerably by gamma irradiation, except the maximum consistency (MC), which decreased at high radiation doses (10 and 15 kGy). Irradiation of semolina also did not significantly affect dough

Table 3

Effect of gamma irradiation on gluten strength of semolina and cooking quality of pasta prepared from four durum wheat varieties

Variety and dose (kGy)	SDS sediment volume (cm^3)	Aleurograph values (bar)			Firmness loss by overcooking (%)
		normal cooking (15 min)	over-cooking (30 min)	total	
GK Basa					
0.0	20.0	63.8	42.4	106.2	33.5
5.0	16.5	62.8	18.6	81.4	70.4
10.0	16.0	18.9	7.0	25.9	63.0
15.0	15.5	38.0	9.2	45.2	75.0
GK Minaret					
0.0	21.0	75.4	44.8	120.2	40.6
5.0	18.5	47.3	21.4	68.7	54.8
10.0	18.0	34.7	13.4	48.1	61.4
15.0	17.0	40.4	15.8	56.2	61.0
Stork-s					
0.0	23.5	71.0	47.5	118.5	33.1
5.0	15.0	66.7	33.2	99.9	50.2
10.0	15.0	53.5	19.3	72.8	63.9
15.0	15.0	57.8	32.8	90.6	43.3
SZD8					
0.0	20.0	64.6	53.4	118.0	16.9
5.0	18.0	66.2	23.6	89.8	64.8
10.0	16.5	39.2	22.4	61.6	42.9
15.0	16.0	36.8	18.8	55.6	48.9
SD 5% between varieties	2.13	13.5	6.96	16.77	14.76
SD 5% between treatments	2.13	13.5	6.96	16.77	14.76
F test for varieties	NS	NS	**	*	NS
F test for treatments	**	**	***	***	**

* Significant at P = 95% probability level
** Highly significant at P = 99% probability level
*** Very highly significant at P = 99.9% probability level
NS: non significant

development time (DDT) in the mixograph test, but significantly decreased MC even at the lowest dose (5 kGy) and area under curve (AUC) at the higher doses (10 and 15 kGy).

The data presented in Table 3 show the effects of gamma irradiation of semolina on gluten strength and cooking quality as determined by SDS sedimentation and aleurograph tests, respectively. Gluten strength of semolina was decreased by irradiation with all doses used. Extensive deterioration of

pasta firmness after normal cooking for 15 min as well as after overcooking for 30 min was caused by gamma irradiation of semolina. The deterioration after normal cooking was significant only at high radiation doses (10 and 15 kGy) except in case of the variety GK Minaret, whereas it became significant even at the lowest dose (5 kGy) after overcooking. Percentage of firmness loss caused by overcooking as an indicator to the tolerance of pasta to overcooking increased considerably under the effect of all radiation doses used.

3. Conclusions

It can be concluded from the results presented in this study that gamma rradiation can be applied to durum wheat semolina for the improvement of pasta colour. The high doses used (5–15 kGy) have improved both pasta colour components, i.e. yellowness and brownness; the former, probably through an effective inactivation of semolina-LPO, the latter, by minimizing the activity of both semolina PO and PPO. On the other hand, gamma irradiation of semolina slightly affected firmness of cooked pasta particularly after overcooking (30 min) and considerably decreased the tolerance of pasta to overcooking. At a dose of 5.0 kGy, pasta colour can be satisfactorily improved, particularly yellowness without significant loss of firmness if the product is cooked carefully and not longer than 15 min.

*

The author wishes to thank Dr. I. Kiss, Head of Microbiology Division, Central Food Research Institute, Budapest for his help in semolina irradiation, and the colleagues of the Cereal Research Institute, Szeged, particulary Ms. E. Ács, Ms. Zs. Kovács for their excellent technical assistance and Dr. F. Sági for revision of the manuscript.

Literature

AACC (1968): Pigment content. American Association of Cereal Chemists, No. 14–50.

Alause, J. & Feillet, P. (1970): Methode semplice obiettivo per la previzione del colore del pasta alimentari. *Tec. molit., 21,* 511–517.

Axford, D. W. E., McDermott, E. E. & Redman, D. G. (1978): Small-scale tests of breadmaking quality. *Milling Feed Fertil., 161,* (5), 18–20.

Badshah, A. & Klopfenstein, C. F. (1989a): Effect of gamma irradiation on the nutritional quality of grain and legumes. — Part I. Stability of niacin, thiamin and riboflavin. *Cereal Chem., 66,* 169–170.

Badshah, A. & Klopfenstein, C. F. (1989b): Effect of gamma irradiation on the nutritional quality of grain and legumes. — Part II. Changes in amino acid profiles and available lysine. *Cereal Chem., 66,* 171–172.

Beczner, J., Kiss, I. & Perédi, J. (1983): Influence of ionizing radiation and storage conditions upon some quality parameters of soybeans. *Acta Alimentaria, 12,* 55–70.

Dexter, J. E. & Matsuo, R. R. (1977): Influence of protein content on some durum wheat quality parameters. *Can. J. Pl. Sci., 57,* 717–727.

Dexter, J. E. & Matsuo, R. R. (1978): The effect of gluten protein fractions on pasta dough rheology and spaghetti-making quality. *Cereal Chem., 55,* 44–57.

DEXTER, J. E., MATSUO, R. R., KOSMOLAK, F. G., LEISLE, D. & MARCHYLO, B. A. (1980): The stability of the SDS-sedimentation test for assessing gluten strength in durum wheat. *Can. J. Pl. Sci., 60*, 25–29.

EL-SAADANY, R. M. A. (1979): Using gamma irradiation for improving rheological properties and baking quality of soft weak wheat flour. *Bakers'Dig., 53*, (4), 34–37.

FEILLET, P. (1983): Present knowledge on biochemical basis of pasta quality: consequences for wheat breeders. *FAO Workshop on Breeding Methology in Durum Wheat and Triticale.* Viterbo. Italy.

FINNEY, K. F. & SHOGREN, M. D. (1972): A ten-gram mixograph for determining and predicting functional properties of wheat flour. *Bakers' Dig., 46*, (2), 32–35, 38–42, 77.

HANIS, T., MNUKOVA, J., JELEN, P., KLJR, P., PEREZ, B. & PESEK, M. (1988): Effect of gamma irradiation on survival of natural microflora and some nutrients in cereal meals. *Cereal Chem., 65*, 381–383.

HONOLD, G. R. & STAHMANN, M. A. (1968): The oxidation-reduction enzymes of wheat. — Part IV. Qualitative and quantitative investigations of the oxidases. *Cereal Chem., 45*, 99–108.

INGRAM, M. & FARKAS, J. (1977): Microbiology of foods pasteurized by ionizing radiation. *Acta Alimentaria, 6*, 123–132.

IRVINE, G. N. (1971): Durum wheat and pasta products. — In: Y. POMERANZ (Ed.) *Wheat chemistry and technology.* 2nd. ed. AACC, pp. 777–797.

IRVINE, G. N. & ANDERSON, J. A. (1953): Kinetic studies of the lipoxidase system of wheat. *Cereal Chem., 30*, 334–342.

IRVINE, G. N. & WINKLER, C. A. (1950): Factors affecting the colour of macaroni. — Part II. Kinetic studies of pigment destruction during making. *Cereal Chem., 27*, 205–218.

IRVINE, G. N., BRADLEY, J. W. & MANTEN, G. C. (1961): A farinograph technique for macaroni doughs. *Cereal Chem., 38*, 153–164.

KOBREHEL, K. & GAUTIER, M. F. (1973): Genetic variability in the peroxidasic composition of wheat. — In: *Proc. S. Genetics and Breeding of Durum Wheat*, Bari, Italy. pp. 527–536.

KOBREHEL, K., LAIGNELET, B. & FEILLET, P. (1972): Relation entre les blés et le brunissement des pates alimentaires *C. r. Akad. Agric. Fr., 58*, 1099–1106.

KOBREHEL, K., LAIGNELET, B. & FEILLET, P. (1974): Study of some factors of macaroni brownness. *Cereal Chem., 51*, 675–684.

KRUGER, J. E. (1976): Change in the polyphenoloxidases of wheat kernel during growth and maturation. *Cereal Chem., 53*, 201–213.

LAIGNELET, B. (1983): Oxidation during the mixing of durum wheat semolina. *Sciences Aliments, 3*, 469–484.

LAMKIN, W. M., MILLER, B. S., NELSON, S. W., TRAYLOR, D. D. & LEE, M. S. (1981): Polyphenol oxidase of hard red winter, soft red winter, white common club durum wheat cultivars. *Cereal Chem., 58*, 27–31.

MATSUO, R. R., BRADLEY, J. W. & IRVINE, G. N. (1970): Studies on pigment destruction during spaghetti processing. *Cereal Chem., 47*, 1–5.

MATVEEF, M. (1966): Influence du gluten des blés durs sur la valeur des pates alimentaires. *Bull. E. F. M., 213*, 133–138.

MCDONALD, C. E. (1979): Lipoxygenase and lutein bleaching activity of durum wheat samolina. *Cereal Chem., 56*, 84–89.

MENGER, A., CLEVE, H. & ANDER, E. (1969): Versuche zur Verbesserung der Teigwarenqualität durch Wärmebehandlung der Getreiderohstoffe. *Brot Gebäck, 9*, 179–183.

SCOTTI, G., BEAUX, Y. & ROBERT, P. (1976): Valeur d'utilisation des blés durs. Une méthode pratique pour l'appréciation de la qualité culinaire. *Bull. E. F. M., 275*, 235–238.

SVÁB, J. (1981): Biometriai módszerek a kutatásban. 3. kiadás. (Application of biometric methods in the research. 3rd ed.) Mezőgazdasági Kiadó. Budapest.

SZÁNTHÓ, P. V., VIGYÁZÓ, V. L. & GYÁRFÁS, F. A. (1981): Determination of lipoxygenase activity in wheat. *Élelmiszervizsg. Közl. 27*, 187–193.

TAHA, S. A. & SÁGI, F. (1986): Relationships between chemical composition of durum wheat semolina and macaroni quality. — Part. I. Total, soluble and insoluble protein. *Cereal Res. Commun., 14*, 259–266.

TAHA, S. A. & SÁGI, F. (1987a): Relationships between chemical composition of durum wheat semolina and macaroni quality. — Part. II. Ash, carotenoid pigments and oxidative enzymes. *Cereal Res. Commun., 15*, 123–129.

TAHA, S. A. & SÁGI, F. (1987b): Quality of durum wheat (Triticum durum Desf.): Grouping of varieties according to their gluten strength, cooking behaviour and gliadin composition. *Cereal Res. Cummun., 15,* 281–288.

URBAIN, W. M. (1986): Radiation chemistry of food components and of foods. — In: SCHWEIGERT, B. S. (Ed.) *Food irradiation, food science and technology.* Academic Press, New York, pp. 37–82.

WALSH, D. E., YOUNGS, V. L. & GILLES, K. A. (1970): Inhibition of durum wheat lipoxygenase with L-ascorbic acid. *Cereal Chem., 47,* 119–123.

WOOTON, M., DJOJONEGORO, H. & DRISCOLL, R. (1988): The effect of gamma-irradiation on the quality of Australian rice. *J. Cereal Sci., 7,* 309–315.

ZACHARIEV, GY. & KISS, I. (1982): The effect of gamma irradiation on some carotenoid compounds of paprika. *5th Symp. on Radiation Chemistry.* Akadémiai Kiadó, Budapest, pp. 1111–1116.

Acta Alimentaria, Vol. 19 (3), pp. 291—293 (1990)

BOOK REVIEWS

Food additives

A. L. Branen, P. M. Davidson Ä S. Salminen (Eds)
Marcel Dekker Inc., New York and Basel, 1990, 736 pages

The 35th volume of a series on food science and technology aims at promoting and enhancing the quality of food products. An overview on the use and consumption of food additives is also included and information is given on the safety evaluation of food additives. The book discusses the main questions in 17 chapters.

The first "Introduction to food additives" gives a comprehensive picture on food additives while enlisting them into 6 main groups: preservatives, nutritional additives, coloring agents, flavoring additives, texturizing agents and miscellaneous additives. A summary of requirements towards additives is given, wholesomeness, economic efficiency and legal questions are dealt with.

The "Estimation of food additive intakes" summarizes the data of examinations performed for the safety utilization of additives; the ADI values and the average and extreme intake in 5 tables. 54 literary references are given.

The "Nutritional additives" deals with pure chemical components like vitamins, amino acids, fatty acids, minerals and trace minerals. Their physico-chemical properties, nutritional- and biological values, isolation, purification and chemical synthesis are also discussed. The chapter is completed with 4 tables and 87 literary references.

The "Antimicrobial agents" chapter discusses the most important components used to protect food from deterioration, their characteristics, possibilities of application, toxicity and authorization. Some of these chemical food preservatives are: bensoic acid and benzoates, sorbic acid and sorbates, acetic acid and acetate salts, lactic acid, propionic acid, fumaric acid, citric acid and miscellaneous acids and further more nitrites, alkyl esters, known as "parabens", sodium chloride, phosphates, hydrogen peroxide, biologically derived antimicrobials, such as for example nisin, natamycin and other inhibitors and other miscellaneous antimicrobials, like carbon dioxide, dimethyl dicarbonate, sucrose esters. Inhibition of microorganisms against the suitable antimicrobial agents is also discussed. 2 tables and 403 literary references complete the chapter.

The "Antioxidants" chapter gives the antioxidants used to keep the quality of food and also to inhibit oxidative rancidity. The phenolic antioxidants: BHA, BHT, alkyl gallates, TBHQ are shown in detail, the natural antioxidant, their origin are surveyed, including the seeds, phospholipids, citrus fruits, amino acids, proteins, reaction products of browning, microorganisms and algae, flavonoids, tocopherols, spices and herbs. As a part of this it deals with the mechanism of oxidation, the increased oxidation inhibition that can be obtained by the combination of antioxidants, the effective utilization of antioxidants according to the types of foods. The authorization of some antioxidants in certain countries is surveyed in tables. The chapter is accomplished by 5 tables and 205 literary references.

The "Flavoring agents" chapter gives an overview of the natural and artificial flavoring agents, on their production, chemical composition and on their areas of utilization. Raw materials, flavoring agents added and their sensory properties are classified. Flavoring agents gained by biosynthesis are given. Purity, quality and identity are emphasized in quality control. The chapter also deals with the function of flavors, with the question of development and safety with the consideration of the standards of Codex Alimentarius. 14 tables detail flavour agents. 60 literary references complete the chapter.

The "Flavor enhancers" deals with the most commonly used substances in this category as monosodium L-glutamate (MSG), disodium 5'-inosinate (IMP) and disodium 5'-guanylate (GMP). The chapter gives a detailed information of the occurence, chemical

properties, stability, industrial utilization of these compounds. Data of toxicological examinations are given and also the knowledge obtained on the fields of acut and chronic toxicity, teratology, mutageneity. The chapter contains 12 tables and 106 literary references.

The "Sweeteners" chapter gives the sugar substitutes in foods. Among the non-nutritive sweeteners it discusses saccharin, sodium cyclamate, aspartame, acesulfame-K, thaumatin compounds and also some sweetening agents localized on other smaller areas, like glycyrrhizin, neosugar, phyllodulcin, miraculin, dulcin, stevioside. Nutritive sweeteners as fructose, sylitol, sorbitol, mannitol, lactitol, lactulose, maltitol, isomalt, fructose syrup and other not at all least important sweeteners are dealt with. The chemical- and technological properties, the questions of toxicity and safety and the regulatory status of the former are shown. 5 tables and 85 literary references complete the textual part.

The "Natural and synthetic coloring agents" chapter states that colorants are very important ingredients in many convenience foods such as confectionery products, gelatin desserts, snacks and beverages, since many of these would be colorless and would thus appear undesirable without the inclusion of colorants. The uses of colorants in the food supply, their chemical properties, safety and governmental regulation are outlined in this chapter.

The "Emulsifiers" chapter surveys the macromolecular emulsifiers and stabilizers. Following compounds are given: lecithin and lecithin derivatives, glycerol fatty acid esters, hydroxycarboxylic acid and fatty acid esters, lactylate fatty acid esters, polyglycerol fatty acid esters, ethylene or propylene glycol fatty acid esters, ethoxylated derivatives of monglycerides, sorbitan fatty acid esters, miscellaneous derivatives. The chemical properties of compounds and the food industrial utilizability by various food types are given, questions of toxicology and regulations are dealt with. 5 tables contain the most important compounds and their properties, 188 literary references complete the chapter.

The "Functions of polysaccharides in foods" chapter describes the structure, function, characteristic properties and usability of polysaccharides. Among them are the starch and the modified starches, glycogen, hemicelluloses, pectin substances, plant gums. 5 tables and 25 literary references complete the chapter.

The "Enzymes" chapter overviews the nomenclature of enzymes, enzyme assays, the functional aspects of enzymes, the mechanism of enzyme action, manufacture of commercial enzymes, use of enzymes in the food industry. This part also deals with toxicity and regulations. 8 figures, 7 tables show the most important data. 144 literary references are given.

The "pH control agents and acidulants" part discusses the physical and chemical properties and chemical analysis of acids, the derivatives of acids and acetate salt that may be given to food. Their use in food is internationally regulated. Organic acids — acetic acid, acetate salts, dehydroacetic acid, diacetate salts apidic acid, ascorbic acid, cyprylic acid, citric acid, fumaric acid, lactic acid, malic acid, propionic acid, succinic acid, tartaric acid — are dealt with.

The antimicrobial effect of the compounds shown are given and also the results of toxicological examinations. The chapter contains 1 table and 137 literary references.

The "Miscellaneous food additives" chapter contains the world-wide used components of "miscellaneous food additives" and their properties: 1. firming agents, 2. formulation aids (binds, carriers, fillers, film-formers, plasticizers), 3. processing aids (anticaking agents, catalysts, crafifying agents and flocculents, clouding agents, filter aids, release agents and libricants), 4. propellants, 5. solvents, 6. synergists. Compounds, their use, regulations, method of examination and toxicity are given in tables. Number of literary references is 43.

The "Methods used in safety evaluation" chapter discusses the requirements under the U. S. Food, Drug and Cosmetic Act that ensure safety of food and color additives, general safety testing and evaluation requirements and procedures, toxicological evaluation of chemicals for carcinogenicity and risk assessment of carcinogenic contaminants in food. The chapter contains 4 figures and 32 literary references.

The "Food additives and hypersensitivity" chapter discusses the questions of the relation between food additives and hypersensitivity in the following details: definitions, mechanism, reaction in the skin, reactions in the airways, other reactions, tests for hypersensitivity reaction (skin tests, oral challenge tests, in vitro tests), prediction of allergy risk, development of tolerance and treatment of hypersensitivity (development of tolerance, treatment of hypersensitivity). The chapter is completed by 12 tables, 9 figures and 207 literary references.

The "Risks and benefits of foods and food additives" chapter survey the risks and benefits of food additives and foods in two parts: "A perspective on risks associated with foods" and in "Categorization of risks and benefits". The risk-benefit and risk-risk approaches to decision making about food additives is dealt with. The part shows the harmful effect of salts, sulfites, saccharin etc. The chapter contains 1 table and 33 literary references.

The book should be useful in theoretical and research work, in solving practical and technological questions and also in post-graduate education.

É. SZÁNTÓ-NÉMETH

Fermentation process development of idustrial organisms

Marcel Dekker, Inc., New York and Basel, 1989; 344 pages

The book is the 4th volume of the "Bioprocess Technology" series.

The 6 chapters discusses with several tables and figures the latest results of strain improvement and fermentation process development of the industrial micro-organisms (antinomycetes, bacteria, coryneform bacteria, fungi and starches) and also that of the tissue cultivation of mammalian cells. The implementation of industrial technology and the questions of rentability are also dealt with. Each chapter forms a separate unit in the above detailed distribution and gives an overall picture on the subjects. Despite of the fact that each chapter was written by an other author, the volume is homogeneous and well-arranged, there is a great number of very well usable literary reference after each chapter.

The book may be a useful handbook for all those industrial experts, researchers and university students who need basic information on the far-reaching fields of bio-technology in the subjects of industrial microbiology, genetical strain improvement, optimation of fermentation processes, scale increase, process technology and production economics.

Á. HOSCHKE

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

ACTA ALIMENTARIA

VOLUME 19 No. 3 — 1990

CONTENTS

Index: 26.039

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1990: Volume 19 (4 issues).
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 19 **December 1990** **Number 4**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

RECENTLY ACCEPTED PAPERS

Infrared spectroscopic study of interactions between phytate and protein in rapeseed
M. KLEPACKA

Water vapour sorption hysteresis and the shelf-life of industrial sponge-cake
GUINOT, P. & MATHLOUTHI, M.

Quality changes of orange soft drinks during storage
VARSÁNYI, I. & SOMOGYI, L.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, B[illegible] and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Review

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 19

1990

AKADÉMIAI KIADÓ
BUDAPEST

CONTENTS

Volume 19

1990

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 19

1990

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 19 (4), pp. 295–304 (1990)

INFRARED SPECTROSCOPIC STUDY OF INTERACTIONS BETWEEN PHYTATE AND PROTEIN IN RAPESEED

M. KLEPACKA

Department of Fermentation and Fruit and Vegetable Technology,
Agricultural University of Warsaw, 02-526 Warsaw,
Rakowiecka 26/30. Poland

(Received: 26 May 1988; revision received: 21 February 1990,
accepted: 26 February 1990)

Extracts (0.2% NaOH), supernatants and isolates were prepared from meals of three varieties of rapeseed (classical, low erucic acid and double improved) and were used to study the interaction between protein and phytates by infrared spectroscopy. Significant differences in the intensity band at 1090 cm^{-1} in the IR spectra of samples were observed. This may be attributed to the effect of the combination of phosphorus with negatively charged proteins. Proteins complexed with phytates appeared both in basic (pH 9.5) and acid environments (supernatants II pH 3.0–4.1).

Keywords: infrared spectroscopy, phytate, rapeseed proteins, interaction between phytate and protein

Phytic acid is the principal source of phosphorus in rapeseed. Phytate phosphorus represents 82–92% of the total phosphorus in rapeseed (UPPSTROM & SVENSSON, 1980). Phytates interact with proteins and form insoluble complexes which under physiological conditions are insoluble (O'DELL & DE BOLAND, 1976; MAGA, 1982; CHERYAN, 1980). The mechanism of reaction between phytic acid and protein depends mainly on pH content of divalent ions and the type of protein (OKUBO et al., 1976; DE BOLAND et al., 1975; REDDY & SALUNKHE, 1981). For studying in the interactions between protein and phytates, various analytical techniques have been applied (O'DELL & DE BOLAND, 1976; SERRAINO & THOMPSON, 1984; REDDY & SALUNKHE, 1981; OKUBO et al., 1976).

In a previous work (KLEPACKA, 1987), attention was paid to the fact in the IR spectrum of the proteins precipitated during heating, the relation between the absorption of the two bands at 1635 cm^{-1} and at 1090 cm^{-1}, respectively, was different and it depended on the protein extracting agent. It was supposed that the band at 1090 cm^{-1} was derived from esters of phosphoric acid associated with protein. The subject of this study was to obtain additional information on the nature of the interaction between protein and phytates by infrared spectroscopy. In the studies the physical and chemical properties of three varieties of rape: classical, low erucic acid and double improved, were compared.

1. Materials and methods

Three varieties of rapeseed were used in this study: classical variety (Górczanski), low erucic acid variety (JG-75), and double improved variety of low glucosinolate and low erucic acid content (MAH-1).

The defatted meals were extracted for 1 h at room temperature with 0.02 *N* NaOH (1:20) at pH 9.5. The slurries were centrifuged at 12.000 x g for 30 min. The supernatants, thus obtained, were referred to as "extract".

On the basis of a previous study (RUTKOWSKI et al., 1985), it was established that a maximum recovery of proteins from the extracts of rapeseed meals might be obtained by a double stepwise precipitation. The extracts were adjusted with HCl to pH 5.5 (classical and double improved varieties) and to pH 4.5 (low erucic acid variety). The solutions were centrifuged. The protein isolate and supernatant, thus obtained, were referred to as "isolate I" and "supernatant I", respectively.

After precipitation at pH 5.5 and 4.5 the supernatants were adjusted with HCl to various pH levels as 3.4: classical variety; 4.1: double improved variety and 3.0: low erucic acid variety, then centrifuged to provide supernatant II and isolate II.

Extracts, supernatants I and II, isolates I and II of the three rapeseed varieties were lyophilized. The samples were analyzed for nitrogen content by the Kjeldahl method. Total phosphorus was determined by the method of FISKE and SUBARROW (1925) and total Ca, Mg, Fe by atomic absorption spectroscopy. The phytates were extracted five times with 1.2% HCl containing 10% Na_2SO_4 for 30 min each. The extracts were pooled and the phytic acid was precipitated as the ferric salt (0.04% $FeCl_3$). The phosphorus content of the insoluble ferric salt was colorimetrically determined after digestion with H_2SO_4 and H_2O_2. The total phosphorus, phytic phosphorus and mineral contents were expressed as g per 100 g nitrogen. In order to understand the nature of protein–phytate complex the effect of $CaCl_2$ and EDTA treatments on the rapeseed meals was determined (SERRAINO & THOMPSON, 1984). Five percent dispersion of rapeseed meals were prepared with $CaCl_2$ (0.25 mol l^{-1}) and EDTA (0.25 mol l^{-1}) and pH was adjusted to 4.5 and 9.5, respectively. All dispersions were stirred for 1 h, centrifuged and the residues were washed several times with distilled water and dried. The infrared experiments were performed using Zeiss IR-75 spectrophotometer by KBr pellet technique. Spectra were recorded for the extracts, supernatants I and II, isolates I and II, residues after $CaCl_2$ and EDTA treated rapeseed meal and ferric phytates.

Polyacrylamide gel electrophoresis for detecting the combination of phytate with protein was used. Samples (extracts, supernatants and isolates) containing about 300–400 μg of soluble proteins were exposed to electro-

phoresis on polyacrylamide gel as described by O'DELL and DE BOLAND (1976). Each sample was loaded on two gels.

After electrophoresis, one of the gels was stained for proteins in 7% acetic acid containing 0.1% Amido Black 10 B and destained in 7% acetic acid and the other one for phytates with ferric chloride (0.074 mol l^{-1} $FeCl_3$ in 0.15 mol l^{-1} HCl, heated at 100 °C for 20 min).

In places where the ferric phytates were present, white bands on orange background were obtained. Densitometric scanning were made with Vitatron TLD 100 densitometer at 546 and 436 nm for gels stained with Amido Black 10 B and ferric chloride, respectively.

2. Results

Figure 1 shows infrared spectra of the examined samples, extract, supernatants (I — at pH 5.5 and II — at pH 3.4) and of isolates I and II. The spectra belonging to the classical variety were presented only, as the example, because in the other varieties, similar dependencies were detected. Between examined samples differences in the intensity of band at 1090 cm^{-1} were observed. In the extracts, supernatants I and II, the bands at 1090 cm^{-1} were more intense than in isolates I and II.

From the examined samples, phytates were isolated and their IR spectra were recorded (not shown). Both bands at 1090 cm^{-1} and at 1700 cm^{-1} were related to the presence of the P=O and C=O and derived from phosphoric esters (WOJTKOWIAK & CHABANEL, 1977).

Table 1 shows the relation between the absorption of the amide band at 1600 cm^{-1} (Amid I) and at 1090 cm^{-1} (P = O). Values obtained in isolates were higher than those in supernatants and extracts which resulted from a lower intensity band at 1090 cm^{-1} in isolates (Fig. 1).

Table 1

Ratio between absorption of Amide I at 1600 cm^{-1} and absorption of the band at 1090 cm^{-1}

Index	Varieties	Samples				
		extract	supernatants		isolates	
			I	II	I	II
$A_{Amide\ I}/A_{1090\ cm^{-1}}$	C	1.16	1.07	1.09	1.36	1.48
	LEAR	1.16	1.11	1.12	1.38	1.38
	DLI	1.19	1.21	1.26	1.96	1.31

Varieties of rapeseed: C — classical, LEAR — low erucic acid content, DLI — double improved

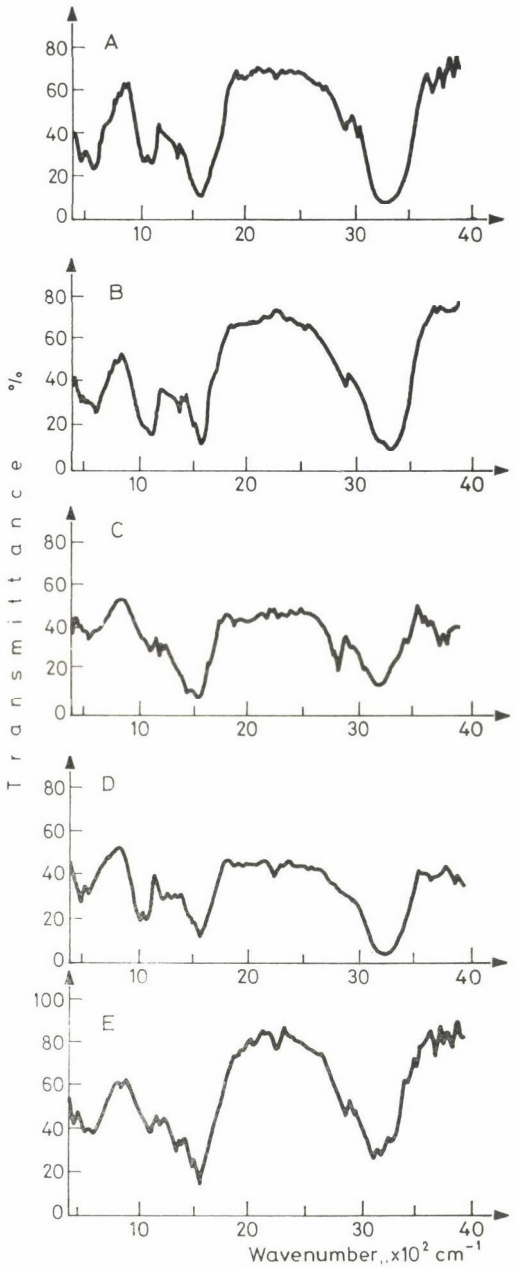


Fig. 1. The IR spectra of the five samples from rapeseed of the classical variety. The samples obtained by the procedures detailed in Chapter 1 were pressed into a pellet with KBr. Symbols: A: extract (pH 9.5); B: supernatant I; C: isolate I; D: supernatant II; E: isolate II

Table 2

Mineral content of the rapeseed meal, extract, supernatants and isolates
(g per 100 g N)

Minerals	Varieties	Samples					
		meals	extract	supernatants		isolates	
				I	II	I	II
Ca	C	3.8	2.1	2.6	2.8	1.1	0.79
	LEAR	8.2	2.8	4.9	5.4	0.82	1.3
	DLI	4.1	2.3	3.6	5.2	0.71	1.4
Mg	C	1.3	1.9	2.3	2.2	0.51	0.39
	LEAR	1.3	2.2	3.9	4.1	0.50	2.7
	DLI	1.1	2.4	5.8	5.0	1.1	2.6
Fe	C	0.42	0.16	0.16	0.12	0.07	0.10
	LEAR	0.28	0.25	0.15	0.20	0.13	0.12
	DLI	0.36	0.18	0.22	0.17	0.14	0.17

Varieties of rapeseed: C — classical, LEAR — of low erucic acid content, DLI — double improved

In order to determine the nature of interaction between phytates and proteins, the rapeseed meal was treated with EDTA and $CaCl_2$. The IR spectra of the residue meals after EDTA or $CaCl_2$ treatments revealed the following relationships between absorption at 1600 cm^{-1} and at 1090 cm^{-1}: 1.32 for EDTA treated meal and 0.96 for $CaCl_2$ treated meal, respectively. The lower value of

Table 3

Total P and phytate P contents of the rapeseed meal, extract, supernatants and isolates

Index	Varieties	Samples					
		meals	extract	supernatants		isolates	
				I	II	I	II
Total P (g per 100 g N)	C	29.0	4.1	3.6	2.9	4.0	2.9
	LEAR	31.1	7.6	8.3	7.7	6.6	9.1
	DLI	29.3	6.2	11.3	9.3	3.7	5.9
Phytate P (g per 100 g N)	C	16.6	1.4	1.1	1.0	1.4	1.4
	LEAR	18.4	2.3	3.0	2.9	3.2	4.4
	DLI	17.5	2.3	3.0	2.1	1.4	3.2
Phytate P of total P (%)	C	57.2	34.1	30.6	34.5	35.0	48.2
	LEAR	59.2	30.2	36.1	37.7	48.5	48.4
	DLI	59.7	37.1	26.5	22.6	37.8	54.2

Varieties of rapeseed: C — classical, LEAR — low erucic acid content, DLI — double improved

the measured ratio of the effect of $CaCl_2$ resulted from the more intensive band at 1090 cm^{-1} in comparison with the EDTA treated sample. The decreased intensity of the band at 1090 cm^{-1} depended on the chelating factor (EDTA) which builds in certain cations more preferentially than phytates and caused disruption of the complex: protein–cation–phytates (CHERYAN, 1980).

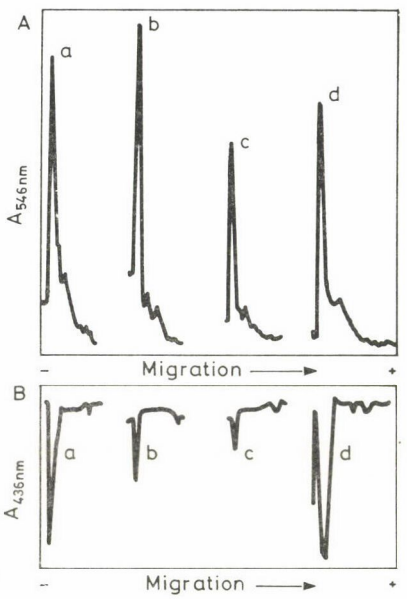


Fig. 2. Densitometric recording of polyacrylamide gel electrophoresis of the proteins from the rapeseed of double improved variety. Symbols: a: extract (pH 9.5); b: supernatant I; c: supernatant II; d: isolate II. The gels designated: A (upper): were stained with Amido Black 10 B and scanned at 546 nm; B (lower): were stained with ferric chloride and scanned at 436 nm. The white bands on orange background, indicating phytate, give decreased densitometric recording. About 300 μg protein were applied to each gel

Table 2 shows the mineral composition of the examined samples, the total contents is expressed as g per 100 g of nitrogen. In the three varieties similar relationships in the quantitative changes of the particular mineral components, were observed. In isolates, the level of Ca and Mg was lowered in comparison to extracts and supernatants. Smaller changes were observed in the Fe content. The differences in phosphorus content, on the other hand, were small, except for the double improved variety (Table 3) which is evidence of a correlation between phosphorus and protein. The level of phytic phosphorus in the studied samples was lower than in the meal. It was observed however, that the phytic phosphorus content in isolates was higher than in supernatants (exception supernatant I of the double improved variety). The phytic phosphorus represents 57–59% of the total phosphorus content in the meal and in the studied

samples it was lower. In isolates II, however, this value was higher (48.2–54.2%) than in supernatants (22.6–37.7%). Between the varieties of rape differences were found in the levels of mineral components.

Interaction between phytate and protein was also examined by polyacrylamide gel electrophoresis. The three varieties of rapeseed have the same bands of electrophoretic separation of the protein from the examined samples. Figure 2 shows, as an example, the results obtained by separation of the proteins from the double improved rapeseed. Each sample was applied to two gels and one of the gels was stained for protein with Amido Black 10 B (Fig. 2A) and the other one for phytate with ferric chloride (Fig. 2B). The electrophoretic patterns of the extract, supernatants I and II were distinct from isolates I and II. The extract, supernatants I and II contained the same major (slow migrating) and two minor (fast migrating) proteins. Since the electrophoretic profiles of isolates I were similar to those of isolates II, therefore Fig. 2 shows results of isolate I, only.

One can see, in Figure 2B, that in all samples the light band in the gel, stained with ferric chloride appeared in the places analogous to the main, slow migrating, protein band.

This observation suggests that only these protein bands were associated with phytates. The differences were observable in the intensities of the bands. Isolate I shows a broader white band than the other samples. The one, small white band noted at the buffer front in all samples indicated that free phytates unassociated with protein, were present.

3. Discussion

The differences occurring between the examined samples in relation to the intensity of the band at 1090 cm^{-1} should probably be ascribed to the combinations of phosphorus and negatively charged substituents. WOJTKOWIAK and CHABANEL (1977) reported that the strongest frequency of band P=O, which derives from phosphoric esters occurs when the substituents combined with phosphorus are negatively charged. Thus there is a close correlation between $\nu_{P=O}$ and the total electronegativity of the substituents. In our experiments the role of these negative substituents may have been played by the proteins, having a negative charge. Proteins, depending on the pH of the environment, have various charges. In an acid environment, below the isoelectric point, proteins possess a positive charge. In a basic environment, above the isoelectric point, protein appears in a proton form and has a negative charge. We concluded that in supernatants (I and II) proteins connected with phytates possessed a negative charge which caused the inrease of the vibration frequency at 1090 cm^{-1} and as a result gave rise to a strong band at 1090 cm^{-1}. Phytic

acid is strongly negatively charged over most of the pH range and the mechanism of reactions with proteins at various pH-s is different (CHERYAN, 1980). At the pH-s below the isoelectric point where protein is positively charged, the binary protein–phytate complex is formed. In pH ranges above the isoelectric point, both, protein and phytic acid are negatively charged and thus the interaction is mediated by multivalent cations to form a ternary protein–cation–phytic acid complex (CHERYAN, 1980). In protein isolates, the considerable reduction on the band at 1090 cm^{-1} probably comes from the fact that protein at the isoelectric point is not charged. A lack of negative change lowers the intensity of this band.

In IR spectra, obtained from the residues after EDTA treatment of the meal a more reduced band at 1090 cm^{-1} was observed than in the $CaCl_2$ treated meal. Hence, it may be assumed that EDTA builds in cations, forming cation–EDTA complex. The disruption of the ternary complex takes place, resulting in lower intensity of the band at 1090 cm^{-1}. $CaCl_2$, on the other hand, acts only on the binary complex and does not disrupt combinations: protein–cation–phytic acid. Hence, the intensity of the band at 1090 cm^{-1} is stronger. The appearance of the stronger band at 1090 cm^{-1} in the supernatant resulted from precipitation of proteins at pH 3.0–4.1 (depending on the variety) and shows that even at this low pH value a part of the rapeseed proteins is negatively charged. Isoelectric points of these proteins appear below pH 3.0. In agreement with LONNERDAL and JANSON (1972) a part of the proteins has isoelectric points near pH 2.0. Appearance of negative charges in the proteins of extracts (pH 9.5) and supernatants I (pH 4.5 and 5.5 depending on the variety) is obvious because proteins of rapeseed have isoelectric points over a wide range of pH. About 20–40% of the proteins have isoelectric points at pH 11.0 and another part at pH 4.0–8.0 (GILLBERG & TORNELL, 1976). Thus, the applied pH conditions during the preparation of isolates, are above their isoelectric points. The combinations of phytates with proteins depend on the type of protein. SERRAINO and THOMPSON (1984) studied interactions of rapeseed proteins with phytates and showed that the binary: protein–phytate complex appeared both at pH 4.0 and at alkaline pH values below pH 11. Also O'DELL and DE BOLAND (1976) report that the formation of complexes between phytates and protein and their interactions with mineral components depend on the origin of vegetable protein.

The variety differences in the level of mineral components in the particular samples as found in the present study, may be connected with various levels of lysine, arginine and histidine. CHERYAN (1980) reported that protein and phytic acid are directly linked through the ε-NH_2 groups of lysine, the guanidine group of arginine and imidazole group of histidine. In an earlier study, it was shown that there are differences in the content of these amino acids between the varieties (NITECKA & KLEPACKA, 1985).

The solubility of complex of phytic acid with mineral components depends mainly on pH. In our study, it was observed that the level of Ca and Mg was considerably reduced in isolates in comparison with extracts and supernatants. In extracts (pH 9.5) the level of Ca and Mg was lower than in supernatants I and II (pH 3.0–5.5). This fact is connected with the solubility of Mg and Ca salts of phytic acid at various pH values. CHERYAN (1980) reports that at higher pH, insoluble salts of Ca and Mg with phytic acid are formed. The solubility of these salts is increased at lower pH values. The level of Fe, on the other hand, did not reveal any significant differences between supernatants and isolates.

The electrophoretic results show that proteins in isolates (I and II) were complexed strongly with phytate, whereas in extract and supernatants only a small part was associated with the protein. The ability of phytic acid to complex protein isolates prepared by isoelectric precipitation is well known (MAGA, 1982; OKUBO et al., 1976; THOMPSON & CHO, 1984).

4. Conclusions

In the IR spectra, the band at 1090 cm^{-1}, which due to the $\nu_{P=0}$ was weaker in the isolates as compared to the extract and supernatants. The phytic phosphorus content in the isolates was high in all the samples and the electrophoretic results support the theory that the phytic phosphorus associated with rapeseed proteins after isoelectric precipitation is strongly bound.

We assume that the negatively charged proteins give rise to a strong band at 1090 cm^{-1}. The negatively charged proteins may appear in combination with phytic acid only in the ternary protein–cation–phytate complex. This complex may be disintegrated using a cation chelating factor e.g. EDTA. In the residues of rapeseed meal after EDTA treatment the decrease of the band at 1090 cm^{-1} was found. The discussed complex appears both in basic environment (extract, pH 9.5) and in acid environment (supernatant II, pH 3.0–4.1) which proves that it is formed by various proteins.

In this study on varieties of rapeseed, the direction of the observed changes in their IR spectra, was similar. This indicates that the genetic removal of erucic acid and glycosinolates has no significant influence on the course of reaction during the combinations between protein and phytates.

Literature

CHERYAN, M. (1980): Phytic acid interaction in food systems. *CRC Crit. Rev. Fd Sci. Nutr.*, *13*, 297–335.

DE BOLAND, A. R., GARNER, G. B. & O'DELL, B. L. (1975): Identification and properties of "phytate" in cereal grains and oilseed products. *J. agric. Fd Chem.*, *23*, 1186–1189.

FISKE, C. H. & SUBARROW, J. (1925): The colorimetric determination of phosphorus. *J. biol. Chem., 66,* 375–400.

GILLBERG, L. & TORNELL, B. (1976): Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. *J. Fd Sci., 41,* 1063–1069.

KLEPACKA, M. (1987): Thermal analysis and infrared spectroscopy study of changes in heat-denatured rapeseed proteins. *Acta Alimentaria, 16,* 53–59.

LONNERDAL, B. & JANSON, J. (1972): Studies on Brassica seed protein. – Part I. The low molecular weight proteins in rapeseed. Isolation and characterization. *Biochem. biophys. Acta, 278,* 175–183.

MAGA, J. A. (1982): Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *J. agric. Fd Chem., 30,* 1–9.

NITECKA, E. & KLEPACKA, M. (1985): Characteristics of seed protein of selected rape varieties. — Part I. Characteristics of albumins of three genetically different rape varieties. *Biul. Inst. Hod. Aklim. Rośl., 158,* 79–89.

O'DELL, B. L. & DE BOLAND, A. (1976): Complexation of phytate with proteins and cations in corn germ and oilseed meals. *J. agric. Fd Chem., 24,* 804–807.

OKUBO, K., MYERS, D. V. & IACOBUCCI, G. A. (1976): Binding of phytic acid to glycinin. *Cereal Chem., 53,* 513–524.

REDDY, N. R. & SALUNKHE, D. K. (1981): Interaction between phytate protein and minerals in whey fraction of Black Gram. *J. Fd Sci., 46,* 564–570.

RUTKOWSKI, A., KLEPACKA, M., NITECKA, E. & GWIAZDA, S. (1985): Usefulness of double-improved winter rapeseeds for getting protein isolates. *Biul. Inst. Hod. Aklim. Rośl., 158,* 49–55.

SERRAINO, M. R. & THOMPSON, L. U. (1984): Removal of phytic acid and protein–phytic acid interactions in rapeseed. *J. agric. Fd Chem., 32,* 38–40.

THOMPSON, L. U. & CHO, Y. S. (1984): Chemical composition and functional properties of acylated phytate rapeseed protein isolate. *J. Fd Sci., 49,* 1584–1594.

WOJTKOWIAK, B. & CHABANEL, N. (1977): *Spectrochimie moléculaire.* Technique et Documentation. Lavoisier, Paris.

UPPSTROM, B. & SVENSSON, B. (1980): Determination of phytic acid in rapeseed meal. *J. Sci. Fd Agric., 31,* 651–656.

Acta Alimentaria, Vol. 19 (4), pp. 305–320 (1990)

OUTLINE OF A SYSTEM FOR THE SELECTION OF THE OPTIMUM STERILIZATION PROCESS FOR CANNED FOODS

PART II. THE DETERMINATION OF HEAT TRANSFER COEFFICIENTS AND HEAT CONDUCTIVITIES IN SOME INDUSTRIAL EQUIPMENTS FOR CANNED PRODUCTS

GY. PÁTKAI, I. KÖRMENDY and M. ERDÉLYI

Institute of Food Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

(Received: 22 March 1989; accepted: 13 February 1990)

The effective heat conductivities in three kinds of baby foods and four kinds of canned meat products, were determined based on heat treatments. The heat transfer coefficients in the divided hydrostatic sterilizers: OHS 6 and OHS 750–26–U_0–M3–102 (made in Hungary) as well as in the horizontal retort Type LW 2002 (without rotations, manufactured by Lubeca, FRG), were also evaluated.

Laboratory experiments were also carried out, where solid metal cylinders of the size of cans and cans filled with boiled egg-white were used.

Results were obtained partly by numerical methods (KÖRMENDY, 1987) based on elementary heat balances, and partly by other, less known, methods or by the expedient combination of the two. The methods are based on the presumption that unsteady-state conditions of heat conduction exist within the canned product. In the course of calculation the thermophysical parameters were systematically varied to achieve between the measured and calculated temperatures the minimum of the mean (square) difference.

Between the heat transmitting medium and the surface of the product the heat transfer coefficient is in the equipment Type OHS 6 $\alpha_R > 200$, in the equipment Type OHS 750–26-U_0–M3–102 $\alpha_R = 500$ and in the horizontal autoclave Type LN 2002 $\alpha_R \approx 700$ $Wm^{-2}\,°C^{-1}$.

The apparent (effective) thermal conductivities varied in canned baby foods between 0.61 and 2.1, in canned meat products between 0.67 and 1.45 $Wm^{-1}\,°C^{-1}$.

In the course of this study the authors took into account the possibility of the development of an optimum-seeking system, too (KÖRMENDY, 1987).

Keywords: thermal conductivity of foods, food sterilization by heat, heat transfer coefficients, numerical methods

Preservation by that treatment is one of the most important methods in the canning industry. The careful selection of the parameters of heat treatment is important for the following reasons: oversterilization reduces the organoleptic and nutritive value of the product, it in volves superfluous consumption of energy and impedes the utilization of the full capacity of the equipment. Inadequate sterilization on the other hand, may cause microbial spoilage. The

establishment of the extent of the necessary and adequate heat treatment may be essentially based on the solution of an optimization task.

The immediate aim of this research work was the establishment of the main physical parameters actually present in the industrial equipment: heat transfer coefficient on the outer surface of a cylindrical can (α_R), apparent (effective) heat conductivity and thermal diffusivity within the product (λ_a, a_a) in the case when inner convection is insignificant. In the foods under examination the convective flow did not extend over the whole mass and only local circulation increases the efficiency of heat transfer.

It is assumed, therefore, that the change of temperature within the canned product is calculable by the method developed for exclusive heat conduction, however, it is calculated with the effective heat conduction coefficient taking into account local convection. Calculations are based on numerical methods developed for the finite and infinite cylinder (KÖRMENDY, 1983, 1987; KÖRMENDY & BORBÉLY, 1985; KÖRMENDY & TOKAJI, 1987) and on the evaluation methods of former experiments (KÖRMENDY & WÜNSCHE, 1983; NAGY, 1987). These permit to compare the results of temperature measurements within the can with calculated values. Systematically varying the thermophysical parameters used in the calculations it is possible to find those values which provide the minimum of the mean difference between measured and calculated temperatures.

The indirect aim of the research work was to use the established thermophysical parameters to calculate in advance the temperature and property fields during heat treatment and to select from the several technological variants the most advantageous one. Another aim of this study was to compare the heat transfer coefficients of some heat treatment equipments used in Hungary.

The symbols used in this paper correspond to those applied by KÖRMENDY (1987), therefore here are only those listed which differ from these.

1. Materials and methods

1.1. The investigated canned products and other materials

1.1.1. Three kinds of baby foods sterilized in jars. The baby foods are listed in Tables 1 and 3. The jar used by the Kecskemét Canning Factory is of 200 cm^3 nominal volume (see Table 2).

1.1.2. Four types of canned meat products as listed in Tables 1 and 3. The cans as follows: ∅ 53-1/10; ∅ 53-1/5; ∅ 99-1/2 in accordance with HUNGARIAN STANDARD (1980).

1.1.3. Boiled white of egg in cans marked 1/5 (see para. 1.1.2.) and in conformity with Tables 1 and 3.

Table 1

Conditions of heat penetration experiments
(Number of parallel measurements are in brackets following serial number)

Serial No.	Material	Type of sterilizer	Type of temperature measuring instrument	Heat treatment equivalent (F_0 min)	Chain speed (m min^{-1})	Temperature of waterbath[a] or formula of sterilization
	Baby foods in jars of 200 cm^3					
1	Puree of French beans+chicken	OHS 6(Kecskeméti Konzervgyár)	Ultrakust	5.9	1.63	M: 85, 95, 105, 112, 118, 126
2	Puree of mixed vegetable+beef		Thermophil	3.5	2.0	G: 130, U: 90, H: 60, SP
3	Pea and potato puree		Store	9.9	2.0	M: 90, 100, 108, 116, 120, 126
4				10.3	2.0	G: 133, U: 90, H: 70, 60 SP
	Canned meat					
5 (3)	Sportspread with cheese 1/10	LUBECA	Ellab	13.9	—	10–30–18/123
6 (2)	Special hashed meat	LW 2002	Cu-constantan	3.8	—	9–35–20/123
7 (3)	Ham salami 1/5			3.0	—	20–75–40/120
8	Veronese hashed meat 1/5			3.0		10–35–20/123
9 (2)	Boiled egg-white in can type 1/5				—	9–35–20/123
10 (2)	Solid Cu cylinder I	LUBECA	Ellab	—	—	10–31–20/123,5
11 (2)	Solid Cu cylinder II	LW 2002	Cu-constantan	—	—	9–35–20/123
12	Solid Cu cylinder	OHS–750–26–$-U_0$–M3–102	Ultracust Thermophil Store		2.2	M: 71, 83, 98, 103, 117, 126, G: 124, U: 90 H: 74, 60, SP, . .
13	Puree of French bean+chicken	Laboratory ultrathermostat	Mercury thermometers	—	—	Heating at constant temperature of 92 °C
14	Puree of mixed vegetable+beef				—	
15	Pea and potato puree				—	
16 (2)	Boiled egg-white				—	

Data of the solid Cu cylinder are given in Table 2

[a] M: hot bath temperatures; G: steam space temperature; U: bath temperature after steam space; H: cooling temperatures, °C; SP: Spontaneously forming temperatures

[b] In the sterilization formula the successive periods, min heating constant temperature, cooling; subsequent to the sign of division: temperature of sterilization, °C

Table 2

Most important parameters of cans, jars, tubes and metal cylinders for running the numerical method to calculate the temperature field

Containers	Inside radius (R)	Height of food filling (H, mm)	Wall thickness (s, mm)	Heat conductivity λ (W m^{-1} °C^{-1})	Number of divisions of nodal points	
					Radius direction (L)	Axis direction (N)
Jars of 200 cm^3 (volume for baby foods)	28.5	75	3.3	0.96	6	4
Comecon-800	46.5	160	4.5	0.96	8	8
∅ 53–1/10 can	26.3	38.1	0.2			
∅ 53–1/5 can	26.3	70.5	0.2	58	6	4
∅ 99–1/2 can prepared from tinned steel plate	49.3	55.0	0.24			
Solid copper cylinders						
1	25.6[a]	39.2			6	4
2	31.15[a]	78.1	no container used		6	4
3	46.5[a]	160.0			8	8
Tube from acid-proof steel plate	20	250.0	1	15	8	—

[a] radius of the superficies of the solid cylinder

1.1.4. Solid copper cylinders in accordance with Tables 1, 2 and 3. The copper used is of high purity, purified electrolytically according to HUNGARIAN STANDARD (1977) (C content above 99.9%).

1.1.5. Materials as specified in paras 1.1.1. and 1.1.3., filled in the metal tube according to Table 2. The tube is sealed and heat isolated at both ends.

1.2. Industrial and laboratory equipments used for heat treatment

Type LW 2002 horizontal autoclave manufactured by Lubeca (FRG), not rotated.

Type OHS-6 divided hydrostatic sterilizer manufactured in Hungary (by the Kiskunfélegyházi Gépgyár) used in the Baby Food Plant of Kecskemét Canning Factory.

Type OHS-750–26-U_0–M3-102 divided hydrostatic sterilizer manufactured in Hungary (by the Kiskunfélegyházi Gépgyár) (operated in the Paks Canning Factory) (SCHMIED, 1988).

Laboratory ultrathermostat, with water circulation of 92 °C at constant intensity.

1.3. Instruments for temperature measurement

Type Thermophil Store 4466 L instrument manufactured by Ultracust (FRG).

Table 3

Composition of the food products and other materials, their thermal and other physical properties at 100 °C

Number	Materials	Total dry matter (%)	Protein (%)	Fat (%)	Specific heat (c) (Jkg °C^{-1})	Density (ϱ) (kg m^{-3})	Thermal diffusivity (a) (m^2 s^{-8} · 10^{-7})	Heat conductivity (λ) (W m^{-10} C^{-1})	Literature
1–13	Puree of French bean+chicken	18.5			3759.7	1048.4	1.47	0.577	CHUBIK & MASLOV (1965)
2–14	Puree of mixed vegetable+beef	14.1			3884.3	1027.7	1.50	0.601	MASLIKOV & MEDVEDEV/1967
3–4–15	Peas and potato puree	14.3			3872.5	1031.4	1.48	0.595	
5	Sportspread with cheese	30	10	10	3488.0	1000.0	1.55	0.540	
6	Special meat hash	40	15	25	3243.0	1007.0	1.52	0.495	CHOI &
7	Ham salami	35	19	16	3319.0	1043.0	1.52	0.523	OKOS (1986)
8	Veronese meat hash	40	15	25	3243.0	1007.0	1.52	0.495	
9–16	Egg-white	15	15		3906.0	1007.0	1.47	0.578	
10–11–12	Solid copper cylinders	—	—	—	383.9	8905.2	1157	395.42	BECKER (1986)

Type CTD–FDQ copper-constant thermocouple manufactured by Ellab (Denmark).

Mercury thermometers with scale division 0.1 °C.

The heat treated materials, their containers, the equipment used for heat treatment and the temperature measuring instruments and their combinations are presented in Table 1.

1.4. Methods for the determination of the thermophysical constants

Thermophysical constants: heat transfer coefficient on the surface of the canned product (α_R), the apparent heat conductivity and thermal diffusivity (λ_a, a_a) in the heat-treated material, they are determined as follows: change of temperature versus time is measured in the geometric center of the tube containing the product or of the metal cylinder and in the heat transfer medium. In the metal cylinders as presented in Tables 2, 3 and 8 the temperature was measured also in a point between the axis and the wall, which point was at the same time on the bisecting plane.

The change of temperature versus time was determined also by calculation for the same point where the inner temperature was measured. Two numerical methods were used for the calculations (one for the finite, the other for the infinite cylinder, KÖRMENDY, 1987). The following data were entered in the calculations: outer temperature changing with time, apparent heat conductivity of the heat-treated product (λ_a), specific heat (c), density (ϱ), thickness of the can wall and its heat conductivity from related literature and the outside heat transfer coefficient (λ, α_R).

In addition the radius (R) of the cylinder pertaining to a can or jar (or just of a cylinder), its height (H), as well as the number of divisions in radial and axial directions needed to the numerical method, were given. These data are given in Table 2.

Temperature calculated as described above and those measured were compared by a method so far known only in a restricted circle (KÖRMENDY & WÜNSCHE, 1983; NAGY, 1987; KÖRMENDY & TOKAJI, 1987).
The equation:

$$\bar{s} = \left(\frac{S}{t_u - t_o}\right)^{\frac{1}{2}} \tag{1}$$

served for calculating the mean (square) difference ($\bar{s}$) between the measured and calculated temperatures. Here

$$S = \int_{t_o=0}^{t_u} (T - T_m)^{2dt} \tag{2}$$

T is the calculated temperature, T_m is the measured one, t is the time, t_0 and t_u are the initial and final points of time of heat treatment in equations 1 and 2.

The calculated values belonging to a given measurement were obtained by the systematically varying of α_R and λ_a (while c ϱ was considered to be constant). To each of the variants the mean square difference was obtained by

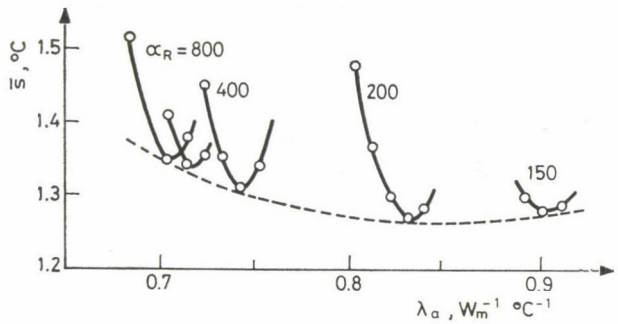


Fig. 1. Graphical determination of the lowest mean difference between the calculated and measured central temperatures as a function of λ_a, for "pea and potato puree" baby food as an example (experiment No. 3)

the use of a computer program (as shown in Fig. 1). Out of these variants those α_R and λ_a values were selected to which the minimum $\bar{s}$ value belongs (marked $\bar{s}_{min}$). The thermophysical parameters thus obtained are shown in Tables 4 and 6.

The initial value of α_R and λ_a was taken from the related literature. The values of the heat conductivities and thermal diffusivities as taken from related literature were marked λ and a.

Appropriate computer programs were developed to the methods as described above, too. The numerical method related to the infinite cylinder was combined with a "minimum of mean difference" selecting process. Thereby α_R and a_a were varied with equal steps within the given domain, all variants evaluate and the one belonging to the minimum of $\bar{s}$ was selected (KÖRMENDY & TOKAJI, 1987). ERDÉLYI (1988) complemented the program of the numerical method related to the finite cylinder with the calculation of the mean deviations, each variant has to be run separately.

These methods, however, did not lead always to satisfactory results. It occurred, namely, that an unrealistically low heat transfer coefficient and an unrealistically high heat conductivity or inversely gave a similar or somewhat smaller average deviation than the best approximations of the "true" values. Therefore, two materials free of local convections were involved in the experiments, the heat conduction coefficients of which were sufficiently well known. With their help first the outer heat transfer coefficient belonging to the given

equipment was determined, then with the fixed value thus obtained the apparent heat conductivity of the food was looked for.

Results of laboratory heat treatment on materials filled in the tube according to para. 1.1.5. were evaluated also by the method of KOPELMAN and PFLUG (1968). The principle of this method is to plit the logarithm of differences between central temperatures of the infinite cylinder and the temperature in its environment versus time. After a short initial phase the relation was found to be

Table 4

Thermophysical properties of baby foods in divided hydrostatic sterilizer (OHS 46)
(Results obtained by the numerical method)

Serial number of experiment	Food product	a_a ($m^2 s^{-1} \cdot 10^{-7}$)	λ_a ($W m^{-1} °C^{-1}$)	α_R ($W m^{-2} °C^{-1}$)	$\bar{s}_{min}$ (°C)	Quotient of apparent and literature values of heat conductivity (λ_a/λ)
1	Puree of French beans + chicken	5.32	2.10	60	0.72	5.64
2	Puree of mixed vegetables + beef	1.63	0.65	700	1.54	1.08
3	Pea and potato puree	2.10	0.83	200	1.27	1.39
4		5.00	2.00	60	0.97	3.36

Average of $\alpha_R = 255$ W m^{-2} °C^{-1}
Standard deviation = 304 W m^{-2} °C^{-1}
Average of $\bar{s}_{min} = 1.12$ °C

Table 5

Physical characters of baby foods as determined in the laboratory in an ultrathermostat
(Evaluation methods: numerical method (1), according to KOPELMAN & PFLUG, 1968 (2))

Serial number of experiments	Food product	Method of evaluation	a_a ($m^2 s^{-1} \cdot 10^{-7}$)	λ_a ($W m^{-1} °C^{-1}$)	α_R ($W m^2 °C^{-1}$)	$\bar{s}_{min}$ (°C)	Quotient of apparent and literature values of heat conductivity (λ_a/λ)
13	Puree of French beans + chicken	1	1.62	0.64	647.5[a]	0.385	1.11
		2	1.50	0.59		—	1.02
14	Pure of mixed vegetables + beef	1	1.7	0.68	533	0.51	1.13
		2	2.1	0.85	197	—	1.40
15	Pea and potato puree	1	2.02	0.82	346	0.50	1.38
		2	2.13	0.85	207	—	1.44

Average $\bar{s}_{min} = 0.47$ °C
[a] Value irrealistically high

Table 6

Thermophysical properties of meat products in the horizontal autoclave, without rotation
(Results obtained by the numerical method)

Serial number of experiment	Serial number of parallel measurement	Product	a_a ($m^2s^{-1}.10^{-7}$)		λ_a ($Wm^{-1}\,°C^{-1}$)		$\bar{s}_{min}$ (°C)		Quotient of apparent and literature values of heat conductivity (λ_a/λ)	
			1[a]	2[b]	1[a]	2[b]	1[a]	2[b]	1[a]	2[b]
5	1 2 3	Sport spread with cheese	1.93	3.02	0.67	1.07	2.29	2.03	1.24	1.98
6	1 2	Special meat hash	1.74	4.45	0.57	1.45	5.16	3.14	1.15	2.93
7	1 2 3	Ham salami	1.27	1.95	0.44	0.67	1.91	1.44	0.84	1.28
8	—	Veronese meat hash	1.42	2.20	0.46	0.72	2.80	1.84	0.93	1.46

1[a] $\alpha_R = 650$, average of $\bar{s}_{min} = 2.9$ °C
2[b] 55 $Wm^{-2}\,°C^{-1} \leq \alpha_R \leq 370$ $Wm^{-2}\,°C^{-1}$, average of $\alpha_R = 199$ $Wm^{-2}\,°C^{-1}$,
standard deviation = 310 $Wm^{-2}\,°C^{-1}$
average of $\bar{s}_{min} = 2.06$ °C

Table 7

Thermophysical properties of egg-white in the horizontal autoclave (without rotation) and in a laboratory thermostat
Methods of evaluation: numerical (1), according to KOPELMAN & PFLUG, 1968 (2)

Serial number of experiment	Serial number of parallel measurement	Equipment	Method of evaluation	a_a ($m^2s^{-1}.10^{-7}$)	λ_a ($Wm^{-1}\,°C^{-1}$)	α_R ($Wm^{-2}\,°C^{-1}$)	$\bar{s}_{min}$ (°C)	Quotient of apparent and literature values of heat conductivity (λ_a/λ)
10	1	LUBECA		1.92	0.873	700	3.69	1.51
	2	LW 2002	—	1.55	0.706	800	7.15	1.22
17	1	Laboratory	1	1.50	0.59	1195	0.39	1.02
		ultrathermostat	2	2.35	0.92	143	—	1.59
	2		1	2.42	0.95	220	0.88	1.64
			2	1.88	0.74	287	—	1.28

linear. Its slope and its intersection with the ordinate give the Biot number and the apparent thermal diffusivity (a_a).

The method is based on the analytical solution of the infinite cylinder's problem, by regarding only the first member of the infinite series. The value α_R is obtained from the Biot number with respect to the heat resistance of the wall

Table 8

Thermophysical properties related to solid copper cylinders in a horizontal autoclave (without rotation) and in a divided hydrostatic sterilizer. Results are obtained by the numerical method. r = the distance of the temperature sensor from the axis of the cylinder in the median plane. r = 0 measurement in the geometric center of the cylinder

Seriel number of experiment	Serial number of parallel measurements	Material		Equipment	Location of temperature measurement (r mm)	α_R (Wm^{-2} $°C^{-1}$)	$\bar{s}_{min}$ (°C)
10	1	Solid copper cylinder	1	LUBECA	0	600	3.00
					16	800	2.46
	2			LW 2002	0	300	10.01
					16	300	9.34
11	1		2		0	900	3.42
					17.95	550	2.52
	2				0	800	0.92
					17.95	800	1.92
12	—		3	OHS 750-26-U_0-M3-102	r=0	500	3.74

(KÖRMENDY, 1987; equations (1) and (2)). The equation of the line was obtained by regression analysis.

The total solids content of the baby foods and egg-white was obtained by drying to constant weight according to HUNGARIAN STANDARD (1971), the composition of the canned meat products was put at our disposal by the Budapest Canning Factory.

The initial thermophysical and other physical characteristics were taken from the related literature. (CHOI & OKOS, 1986; CHUBIK & MASLOV, 1965; MASLIKOV & MEDVEDEV, 1967).

The protein, fat and water contents of the meat products, the total solids content of baby foods and egg-white were substituted in the equations of the related literature (Table 3).

The experiments were generally carried out in 2–3 parallel measurements (Table 1).

2. Results

Conditions of the measurements are contained in Table 1. The geometrical data needed to the evaluation are listed in Table 2, the composition and the initial thermophysical and other physical data in Table 3.

Data on baby foods are given in Tables 4 and 5, the results related to canned meat products in Table 6, those related to the egg-white and the solid copper cylinders in Tables 7 and 8.

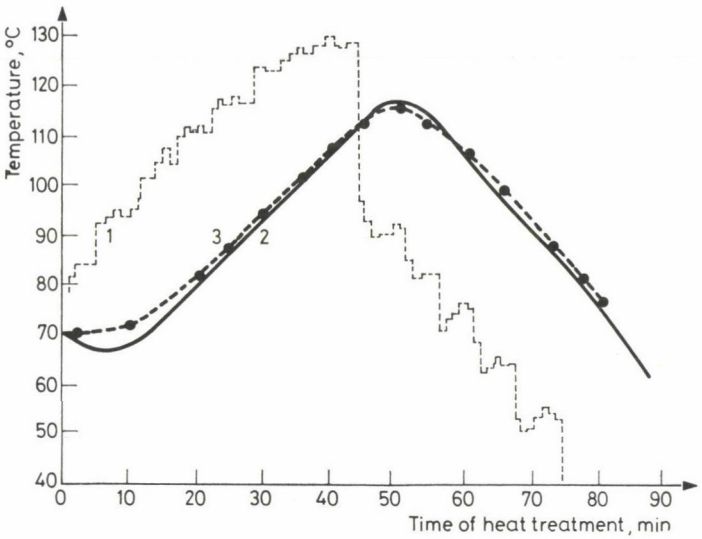


Fig. 2. Measured and calculated values in a heat penetration diagram based on the heat treatment of "Puree of mixed vegetables and beef" baby food in Type OHS-6 equipment (experiment No. 2) as an example. – – – – –: Temperature of water bath; ———: central temperature as measured; ●– – – –●: central temperature as calculated

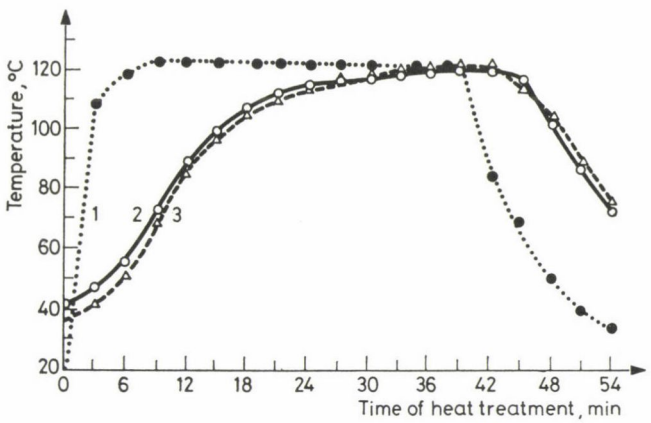


Fig. 3. Measured and calculated values in a heat penetration diagram based on the sterilization of "Sport spread with cheese" as an example (experiment No. 5). ●····●: Calculated value; ○——○: measured value; △– – – –△: water bath

As an example, in Figs 2 and 3 the temperature as measured and calculated in a divided hydrostatic sterilizer and in a horizontal autoclave are illustrated as functions of time.

The data of experiment of serial number 3 in Table 1 were taken from the diploma thesis of SZATMÁRI-NAGY (1984) and were evaluated here.

3. Conclusions

3.1. Baby foods

It is seen in Table 4 that the temperature as measured in the geometric center of the jars can be approximated by the numerical method very well. ($0.72 \leq \bar{\delta}_{min} \leq 1.54$ °C). The outer heat transfer coefficient and the apparent heat conductivity, however, vary between very large limits:

$$60 \leq \alpha_R \leq 700 \text{ Wm}^{-2} \text{°C}^{-1}, \; 0.65 \leq \lambda_a \; 2.1 \text{ Wm}^{-1} \text{°C}^{-1}, \; 1.08 \leq \lambda_a/\lambda \; 3.64.$$

It can be seen further, that to a high α_R belongs a low λ_a and to a low α_R a high λ_a. The effect of an irrealistically low heat transfer coefficient can be compensated in the calculations by an irrealistically increased heat conductivity and in this case the mean difference of the measured and calculated temperatures is possibly lower than the difference obtained by reasonable data. In Table 4 the experiments of serial number 2 gave reasonable results.

The laboratory measurements evaluated either by the numerical method or according to KOPELMAN and PFLUG (1968) gave substantially better results. According to the numerical method the mean value of α_R is 491.6 $\text{Wm}^{-2} \text{°C}^{-1}$ and the standard deviation around the mean is 210 $\text{Wm}^{-2} \text{°C}^{-1}$ (based on the parallel measurements). Both α_R and λ_a varies in a much narrowed region. The average standard deviation of λ_a around the mean is 0.10 $\text{Wm}^{-1} \text{°C}^{-1}$.

Evaluating according to KOPELMAN and PFLUG (1968) the mean value of α_R amounts to 437 $\text{Wm}^{-2} \text{°C}^{-1}$ with a standard deviation of 609 $\text{Wm}^{-2} \text{°C}^{-1}$. These are, however, unreliable results because they were formed by omitting the results of one of the parallel measurements ($\alpha_R \to \infty$ in experiment No. 13). The scatter of λ_a around the mean of parallel measurements is 0.05 $\text{Wm}^{-1} \text{°C}^{-1}$.

On comparing the two methods of evaluation related to λ_a by one-way hierarchical analysis of variance (hierarchical levels: product, method of evaluation, parallel measurements) the following were found: with two products (Nos 13 and 14) the difference between the two methods was significant, while with the third product (No. 15) the difference was non-significant.

The two methods of evaluation were compared also by testing the mean difference of paired data of results related to α_R and λ_a (SVÁB, 1981).

Scatter of the mean difference of paired data of calculated from the same experiment by two different methods amounts to 0.04 $\text{Wm}^{-1} \text{°C}^{-1}$, the significant difference is 0.09 $\text{Wm}^{-1} \text{°C}^{-1}$, the value of the mean difference is 0.07 $\text{Wm}^{-1} \text{°C}^{-1}$.

Therefore no significant difference was found between the values as obtained by the two methods, when the three experiments were examined together.

It is interesting to note that the numerical method resulted in a lower scatter of α_R, where according to the method of KOPELMAN and PFLUG (1968)

the scatter of λ_a was less. If the mean values of λ_a for each product separately in Table 5 are used to recalculate the respective α_R values for the equipment Type OHS 6 then $\alpha_R \geq 200$ $Wm^{-2}\,°C^{-1}$ values are obtained. The mean values of λ_a of the products in experiments 13, 14 and 15, respectively, are as follows: 0.62, 0.77 and 0.84 $Wm^{-1}\,°C^{-1}$.

3.2. Canned meat products

The industrial experiments related to canned meat products permit similar conclusions (Table 6) as the baby foods (para 3.1.,Table 4). However, the scatter of individual values is lower but the mean difference is about the double of that of the baby foods ($1.44 \leq \bar{s}_{min} \leq 3.14$ °C).

The heat transfer coefficient is: $55 \leq \alpha_R \leq 370$ $Wm^{-2}\,°C^{-1}$, the heat conductivity is: $0.67 \leq \lambda_a \leq 1.45$ $Wm^{-1}\,°C^{-1}$, λ_a/λ varied between the limits: $1.28 \leq \lambda_a/\lambda \leq 2.93$. Probably the reason for lower fluctuation of the values is due to the fact that in each 2–3 parallel measurements were carried out. Scatter of the parallels of λ_a around the means of the products was 0.29 $Wm^{-1}\,°C^{-1}$.

In accordance with previous considerations the value of α_R can be also determined from experiments carried out with high purity copper cylinders (para. 3.4., Table 8). The result of these experiments was a heat transfer coefficient of 631.25 $Wm^{-2}\,°C^{-1}$ on the average (values of a and λ are reliable data from related literature) with a standard deviation of 234 $Wm^{-2}\,°C^{-1}$. For reasons of calculation technique a value somewhat higher, $\alpha_R = 650$ $Wm^{-2}\,°C^{-1}$ was selected and the numerical method was repeated with canned meat products. Results can be seen in Table 6. The fluctuation of values of λ_a diminished substantially. These values already approximate the data in the literature, while the value of $\bar{s}_{min}$ slightly increased. Scatter of λ_a around the means of individual product was: $2.48 \cdot 10^{-2}$ $Wm^{-1}\,°C^{-1}$.

3.3. Boiled egg-white

Results of the experiments with boiled egg-white are shown in Table 7. In the horizontal autoclave (without rotation) the average heat transfer coefficient was 750 $Wm^{-2}\,°C^{-1}$ (para. 3.4.). In the laboratory ultrathermostat the average value of λ_a was 461.25 $Wm^{-2}\,°C^{-1}$ and this is in good agreement with the value obtained for baby foods (para. 3.1., Table 6).

Average value of λ_a was 0.797 $Wm^{-1}\,°C^{-1}$, its scatter 0.14 $Wm^{-1}\,°C^{-1}$. On the basis of analysis of variance the values of λ_a for the two experiments can be averaged contracted. The average value of λ_a divided by the value found in the literature makes 1.38. It should be noted however, that the data in the literature do relate to unboiled egg-white.

3.4. Solid copper cylinders

Experimental results related to the copper cylinders are shown in Table 8. Data pertinent to a horizontal autoclave were analyzed by one-way hierarchical analysis of variance (levels of hierarchy: type of the copper cylinder, parallel measurements, location of temperature measurement in the solid cylinder). Conclusion was that the averages belonging to the groups of individual levels cannot be separated, the overall mean of the eight data could be constituted ($\alpha_R = 631.26$ Wm^{-2} $°C^{-1}$). Although the effect of type of copper cylinder and randomness of location in the retort parallel measurement) could not be demonstrated, results make it probable that these effects can be (partly) separated by increased number of measurements. Data pertinent to the location of temperature measurement showed the least fluctuation, α_R corresponded in two cases out of four.

In the apparatus of Type OHS 750–26–U_0–M3–102 the solid copper cylinder gave the value $\alpha_R = 500$ Wm^{-2} $°C^{-1}$.

Measurement with copper cylinder has two defects: it takes over the environmental temperature too quickly; the temperature of the surface differs from that of the food product, namely the value of α_R may depend on the difference in temperature between environment and surface, too.

3.5. Summarized discussion

From the experiments the following can be concluded: with the help of the numerical temperature calculation method based on elementary heat balances the heat penetration diagrams can be very well approximated.

Using a λ_a value standing near to data in the literature the process resulted mostly in a mean difference of $\bar{s}_{min} < 2$ °C in comparison to measured values (Tables 4, 5, 6, 7 and 8). Only the experiments of serial numbers 6, 10 and 11 form exception.

Thus the numerical calculation method used in this work is suitable to calculate the temperature field forming during heat treatment in the canned product.

The comparative analysis of the property values mainly the F_0 value based on measurement and calculation is a further task and is inevitably necessary to the selection of the most favourable technological variant.

The comparison of values obtained by measurement and calculation by the numerical method in the can's centre is less suitable for the determination of α_R because the temperature calculated for the center is not sensitive to variations in α_R. The curve (Fig. 1) connecting the minimum values of functions $s = f(\lambda_a)$ belonging to constant α_R is of a flat shape. The location of its minimum may be more affected by temperature measurement error or by inaccuracy of positioning the temperature sensor, than by changes in α_R.

It is recommended for the determination of α_R by the numerical method to measure the temperature either on the heat transfer surface or in a point near to it in further experiments.

In addition to the above conclusions: to be able to determine α_R accurately the exact knowledge of the heat conductivity of the material used for measurement is also needed. It is suggested to use in further experiments of this character materials the heat conductivity of which is similar to that of foods and are free of local convection.

This value of $\bar{s}$ is much more sensitive to changes in λ_a, particularly in the range of α_R occurring in canning practice. Using α_R previously determined this method provided results approximating λ_a values of related literature (Table 8).

The way in which heat treatment and temperature measurement are carried out in the laboratory influenced substantially the accurracy of λ_a. Both λ_a and λ_a/λ fluctuate in a significantly narrower region than with measurements carried out in industrial equipments (see Tables 4 and 5).

In the previous knowledge of α_R and with exact measurement of temperature the numerical method can be proposed for the determination of λ_a, primarily, if data of heat penetration measurements originate from laboratory conditions. The computer program developed for the case of infinite cylinder permits particularly rapid and convenient evaluation.

Symbols

The majority of symbols used in this paper can be found in the paper of KÖRMENDY (1987), here the complementary list of symbols is given only.

a: Thermal conductivity (from literature, $m^2 s^{-1}$)

a_a: Effective (apparent) thermal conductivity ($m^2 s^{-1}$)

F_0: Heat treatment equivalent $T_r = 121.11 \ldots$ °C, $z = 10$ °C (min)

r: Distance from the axis in the median plane (mm)

$\bar{s}$: Mean (square) difference in temperature between the values measured and calculated (°C)

$\bar{s}_{min}$: minimum of $\bar{s}$ (°C)

S: Value of the definite integral see equation (2), °C² min applied to the calculation of the mean temperature difference

$t, t_0 t_u$: Heat treatment time, the initial and final point of time (°C)

λ: Heat conductivity (from the literature, $Wm^{-1} °C^{-1}$)

λ_a: Apparent (effective) heat conductivity ($Wm^{-1} °C^{-1}$)

*

The authors wish to express their thanks to Ms. L. MOSKOVICS, head of the technology department (Budapest Canning Factory) for her efficient participation in the experiments, to Mr. G. TOKAI group leader (Central Office, Hungarian Academy of Science) for programming, to Mr. L. MÉSZÁROS and Dr. B. ZÁMBO engineers (University

of Horticulture and Food Industry) for their expert advice and to Prof. Sz. TÖRÖK (University of Horticulture and Food Industry) for promoting this work and guaranteeing the proper conditions.

Literature

BECKER, M. (1986): *Heat transfer*. Plenum Press, New York, London.

CHOI, Y. & OKOS, M. R. (1986): Effects of temperature and composition on the thermal properties of food. — In: LA MAGUER, M. & JELEN, P. *Food engineering and process applications*. Elsevier Applied Science Publishers, London, Vol. 1, pp. 93–101.

CHUBIK, Y. A. & MASLOV, A. M. (1965): Spravochnik po teplofizicheskim konstantam pischevyh produktov i polufabrikatov. *Pishch. Tekhnol.*, *2*, 79–92.

ERDÉLYI, M. (1988): Personal communication.

HUNGARIAN STANDARD (1971): *Tartósított élelmiszerek szárazanyagtartalma*. (Solid content of foods.) MSZ 3607.

HUNGARIAN STANDARD (1977): *Réz. Anyagminőségek*. (Copper. Quality of materials.) MSZ 64/1–77.

HUNGARIAN STANDARD (1980): *Konzervdobozok*. (Cans for preserved foods.) MSZ 08–1467–80.

KOPELMAN, I. J. & PFLUG, I. J. (1968): The relationship of the surface, mass average and geometric center temperatures in transient conduction heat flow. *Fd Technol.*, *22*, (6), 141–146.

KÖRMENDY, I. (1983): A véges henger hőkezelése midőn a belső hőközlés kizárólag hővezetéssel megy végbe. Az EMG 666/B típusú (magyar) számítógépre írt program dokumentációja. (Heat treatment of the finite cylinder when the inside heat transfer is carried out only by heat conduction. Documentation of a computer program prepared for Type EMG 666/B computer (Hungarian). Kertészeti és Élelmiszeripari Egyetem, Konzervtechnológiai Tanszék, Budapest.

KÖRMENDY, I. (1987): Outline of a system for the selection of the optimum sterilization process for canned foods. — Part I. Calculation methods. *Acta Alimentaria*, *16*, 3–27.

KÖRMENDY, I. & BORBÉLY, A. (1985): Hőmérséklet- és tulajdonságmező számítása a véges hengerben, kizárólagos hővezetés esetében. Programdokumentáció Commodore 64 számítógépre. (Calculation of the temperature and parameter field in a finite cylinder, in the case of exclusive thermal conduction. Documentation of a program for Commodore 64 computer.) Kertészeti és Élelmiszeripari Egyetem, Konzervtechnológiai Tanszék, Budapest.

KÖRMENDY, I. & TOKAJI, G. (1987): Meancalc és Heatcalc című programok dokumentációja, IBM kompatibilis számítógépre. (Documentation of the programs prepared for IBM compatible computer: Meancalc and Heatcalc.) Kertészeti és Élelmiszeripari Egyetem, Konzervtechnológiai Tanszék, Budapest.

KÖRMENDY, I. & WÜNSCHE, H. (1983): Osztott hidrosztatikus sterilező berendezés hőkezelési viszonyainak elemzése lecsókonzerv hőkezelésénél. (Analysis of the heat treatment conditions in a divided hydrostatic sterilizer with heat treatment of paprika in tomato juice.) *Konzerv Paprikaipar*, (2), 60–64.

MASLIKOV, V. A. & MEDVEDEV, O. K. (1967): Nekotorye fizicheskie konstanty tomato productov. *Pishch. Tekhnol.*, *4*, 78–79.

NAGY, L. (1987): 5/1 dunsztalma hőkezelése, a hőbehatolás mérése és számítása. Szakdolgozat. (Heat treatment of canned apple in syrup, measurement and calculation of the inside temperature in cans of type 5/1.) B. Sc. thesis. Kertészeti és Élelmiszeripari Egyetem, Budapest.

SCHMIED, J. (1988): A Hunister legújabb típusa: OHS–750–26–U_0–M3–105. (Latest type of the HUNISTER sterilizer: OHS-750-26-U_0-M3-105.) *Konzerv Paprikaipar*, (1), 8–13.

SVÁB, J. (1981): *Biometriai módszerek a kutatásban*. (Methods of biometry in research work.) Mezőgazdasági Kiadó, Budapest, pp. 53–55.

SZATMÁRI-NAGY É. (1984): Kecskeméti Konzervgyárban gyártott bébiétel konzervek kritikai vizsgálata. (Critical study of baby foods manufactured in the Kecskemét Canning Factory.) Kertészeti és Élelmiszeripari Egyetem, Budapest.

Acta Alimentaria, Vol. 19 (4), pp. 321–329 (1990)

STUDIES ON THE SHELF LIFE OF MODIFIED CAMEMBERT CHEESE

P. PALICH, W. DERENGIEWICZ[a] and J. SWITKA

Merchant Marine Academy, Department of Catering, 81—962 Gdynia ul. Czerwonych Kosynierów 83. Poland

[a]Cooperative Dairy, Turek. Poland

(Received: 3 October 1989; accepted: 22 March 1990)

The paper presents investigations of the influence of temperature on the storage life of Camembert cheese as well as cheese manufactured by a modified production process.

The cheeses were examined for pH, acidity, moisture, soluble nitrogen, nonprotein nitrogen and peptide nitrogen content, free fatty acids content and maturity degree. Changes in organoleptic quality (taste and smell, shape and appearance, consistence, colour) were evaluated by taste panel. Classification and quantification of critical characteristics have been made and mathematical models of the investigated changes and shelf life of the tested cheeses have been established. The quality changes can be described with quadratic function. The accuracy of curve fitting was characterised by the correlation coefficient.

The shelf life of modified Camembert cheese stored at temperature of 4 °C was found equal to 28 days while stored at temperature of 18 °C was found equal to 6 days. Compared to the standard Camembert cheese this was longer by 7 days and by 3 days, respectively.

Keywords: Camembert cheese, critical characteristics, shelf life

Soft cheeses with surface mould belong to a group of cheeses with high nourishing and taste quality properties. The high biological and dietetic value of these cheeses, as well as social and economical factors, encouraged expansion of their production and consumption (POZNAŃSKY & SIUDAK, 1971; POZNAŃSKI et al., 1979; ŚMIETANA et al., 1973).

Such cheeses, however, have relatively short shelf lives which depend also on the storage and distribution conditions. The principle of continuous cooling should be observed during the distribution and sale of such types of cheese (STEHLE, 1980). However, this principle is not usually followed due to various reasons, and therefore, the quality of cheeses decrease fairly quickly.

Besides continuous cooling of a ripened cheese there are sereval technical and economical factors of importance during the product on process, which considerably influence cheese quality and shelf life (KAMMERLEHNER&KESSLER, 1982; STEHLE, 1980).

Modification of some of the technological parameters of Camembert cheese production changes its chemical composition as well as its maturation process and shelf life.

Many authors (LABUZA & RIBOH, 1982; LENZ & LUND 1980; MISHKIN et al., 1984) are of the opinion that almost all reactions taking place in the

food are of the first or zero order, therefore they can be described by means of a linear, quadratic or exponential function. Description of variations of investigated factors taking place in the food when stored by a mathematical function enables the creation of mathematical models of these variations.

Derivation of mathematical models describing changes of quality characteristics taking place in food products simplify to a great extent their prediction. Application of such formulae enables limitation of time-consuming analysis. To facilitate prognosis, very often critical characteristics representing a rate of product quality decrease is calculated.

The aim of the present work was to classify critical characteristics, to quantify their values, and to establish a mathematical model of their variations and the shelf life of Camembert cheese as well as cheese produced according to a modified production process.

1. Material and methods

1.1 Material

Soft cheese with surface mould was investigated: Camembert cheese and cheese produced according to a modified process.

Production of cheese according to the modified manifacture process differs from that of Camembert cheese by a higher grade of milk souring (8.5 °SH), increased addition of rennin, cutting the curd into smaller cubes (1 cm^3), replacement of about 30% of the whey by pasteurized and cooled (to 50 °C) water, thus increasing the temperature of cheese slurry up to 37–38 °C. Also, the time of cheese ripening has been prolonged up to 20 days.

Cheese manufactured according to the modified production process was then stored at 18 °C and 4 °C. Organoleptic, physical and chemical analyses of the fresh cheese and during its storage were made. Quality and shelf life of the cheese manufactured according to the modified production process were compared with that of standard Camembert, fresh and stored under the same conditions.

1.1.1. Preparation of samples for analysis. Each new series of tests were started with an organoleptic assessment. In order to obtain uniform samples, cheese was milled before physical and chemical analysis. During the analysis period, the samples were kept in glass jars at 4 °C.

1.2. Methods

Five panelists were employed to carry out organoleptic examination using a 5 points scale. Coefficients of weight ability of investigated characteristics were established by the panel on the basis of the methods published by Ba-

RYŁKO-PIKIELNA (1985). The following coefficients were accepted: shape and appearance — 0.15, structure and consistence — 0.25, colour — 0.1, taste and smell — 0.5.

Further investigations were made by methods given by BUDSŁAWSKI (1972): acidity, pH, moisture content, fat, content of nitrogen compounds; total content of soluble nitrogen compounds; content of nonprotein compounds. Content of nitrogen peptide compounds was determined by means of BOULANGER's method (1949), maturation degree by means of SCHNEIDER's and ROEDEL's method (1979) and content of free fatty acids by DEETH's and co-workers' method (1976).

Changes in the quality of the cheeses during storage were determined on the basis of acidity, soluble nitrogen compounds, maturation degree and content of free fatty acids.

Results obtained are presented in the form of tables and figures. For the investigated quality factors of the cheeses, the following coefficients were calculated: arithmetic average, standard deviation and coefficient of variation. The influence of temperature and storage time on quality characteristics was determined on the basis of linear correlation coefficients, while their dynamics and the choice of mathematical model best describing the changes are presented in the form of table and figure. Approximation of model has been carried out by means of the least squares method, using the curve of the smallest mean square sum of deviations between empirical and theoretical values and essential correlation coefficient (level of confidence 0.05). Significance of correlation coefficients has been found by verification of the hypothesis on the correlation lack by means of t-Student rest (BOZYK & RUDZKY, 1977).

2. Results and discussion

Introductory organoleptic evaluation of cheese showed that there are quality factors typical for Camembert cheese, and decisive for its high quality.

On the other hand, cheese manufactured according to the modified production process showed a more compact, elastic and uniform consistence within the whole substance, however without the core characteristic typical of Camembert cheese. During storage the highest dynamic of organoleptic properties, changes of taste and smell, for both types of cheese were noted. So, these characteristics were accepted as critical. The following were unacceptable quality factors: unpleasant, spicy and bitter flavour and the smell of ammonia. Such characteristics for Camembert cheese stored at 18 °C or 4 °C have been found after 3 and 21 days, respectively, while for the cheese manufactured according to modified production process after 6 and 28 days, respectively. The critical value of cheese quality factors in organoleptic evaluation was the value of 3

points. Cheese of lower than 3 point value in total organoleptic assessment were disqualified (Fig. 1).

Modification of the production process caused distinctive differentiation of physical and chemical properties of cheese (Table 1). Comparison of Camembert cheese and modified cheese showed, that the latter was of lower acidity

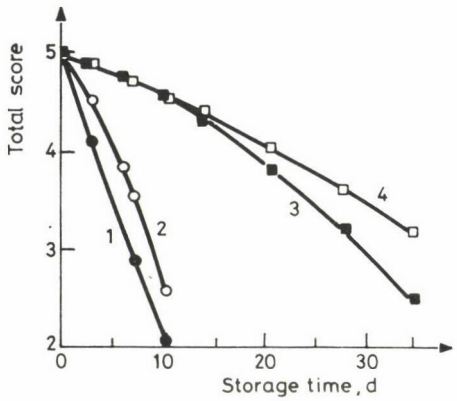


Fig. 1. Changes of total score of organoleptic evaluation of Camembert and modified cheese during storage. ●——●: Camembert (18 °C); ○——○: modified (18 °C); ■——■: Camembert (4 °C); □——□: modified (4 °C)

and moisture content. However, the content of nitrogen compounds was slightly higher. On the other hand, the maturity degree, indicating the intensity of proteolytic alteration and the content of free fatty acids, was found to be higher, thus reflecting the intensity of lipolytic alterations (Table 1).

Table 1

The physicochemical characteristics of matured Camembert cheese and cheese produced according to the modified process

Characteristics	Camembert			Modified cheese		
	$\bar{x}$	±s	v	$\bar{x}$	±s	v
Acidity (°SH)	66.00	2.85	4.31	52.00	2.45	4.71
pH	4.37	0.20	6.17	4.86	0.16	3.29
Moisture (%)	51.52	1.96	3.80	45.46	1.56	3.43
Fat (%d.m.)	46.41	1.45	3.12	45.21	1.35	2.98
Total nitrogen (%d.m.)	6.87	0.37	5.38	7.17	0.47	6.55
Soluble nitrogen (%d.m.)	1.80	0.06	3.33	2.68	0.07	2.61
Nonprotein nitrogen (%d.m.)	0.25	0.03	12.00	0.27	0.06	22.22
Peptides nitrogen (%d.m.)	0.06	0.02	33.00	0.06	0.02	33.00
Maturity degree	40.00	2.53	6.32	40.00	2.35	5.87
Free fatty acids (μEq/g)	11.32	0.93	8.20	11.00	0.73	6.63

Number of measurements ($n_1 = n_2$) = 8
$\bar{x}$: mean value, s: standard deviation, v: variation coefficient (%)

Table 2

Mean values of cheese characteristics at the disqualification point

Characteristics	Sample			
	A	B	C	D
Acidity (°SH)	49.00	43.00	33.50	30.00
pH	5.89	5.76	6.17	6.54
Soluble nitrogen (% d.m.)	4.04	4.58	4.38	4.86
Maturity degree	73.00	71.00	82.00	87.00
Free fatty acids (μEq/g)	29.20	28.13	19.24	18.33
Shelf life (days)	3	6	21	28

A: Camembert stored at 18 °C
B: modified cheese stored at 18 °C
C: Camembert stored at 4 °C
D: modified cheese stored at 4 °C

Statistical estimations of the results in Table 1 showed their considerable dispersion for some characteristics. Therefore, when discussing changes due to storage, mainly characteristics of lower than 10% coefficient of variations were taken into account. Characteristics, that showed relatively constant volumes i.e. the moisture content, fat and total nitrogen compounds were not discussed.

During storage a high dependence and correlation coefficients between storage time and changes of investigated characteristics was found in the case of the acidity and pH, content of soluble nitrogen compounds, rate of ripening and content of free fatty acids (Table 2).

Acidity (pH) of cheeses kept at 18 °C as well as content of soluble nitrogen compounds considerably increased and higher dynamics of changes were found in the Camembert cheese. A similar tendency, however of much lower dynamics, were noted in cheese stored at 4 °C (Figs. 2 and 3). The very different critical values of the above-mentioned characteristics indicate their different dynamics, which depend on storage as well as on the kind of cheese (Table 2). Only in the case of soluble nitrogen compounds were the values found to be similar. Their content in Camembert cheese stored at 18 °C was found to be 4.04% in dry matter, and in cheese manufactured according to the modified process, 4.58%. On the other hand cheese stored at 4 °C showed values 4.38% and 4.86%, respectively, in dry matter (Table 2). Thus, this characteristic has been accepted as critical, while a critical value of useability for consumption equal to 4.5% of nitrogen compounds soluble in dry matter has been accepted. On the basis of the results it was concluded that the optimal storage period for high quality Camembert cheese stored at 18 °C amounts to 3 days, and of cheese manufactured according to modified production process amounts to 6 days. Decrease of storage temperature to 4 °C extended these times to 21 and 28 days, respectively. Similar results of dependence between storage temperature and shelf

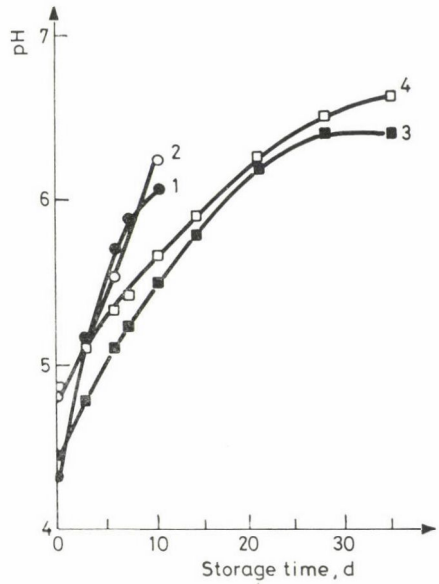


Fig. 2. The pH changes in Camembert and modified cheese during storage at 18 °C and 4 °C. ●——●: Camembert (18 °C); ○——○: modified (18 °C); ■——■: Camembert (4 °C); □——□: modified (4 °C)

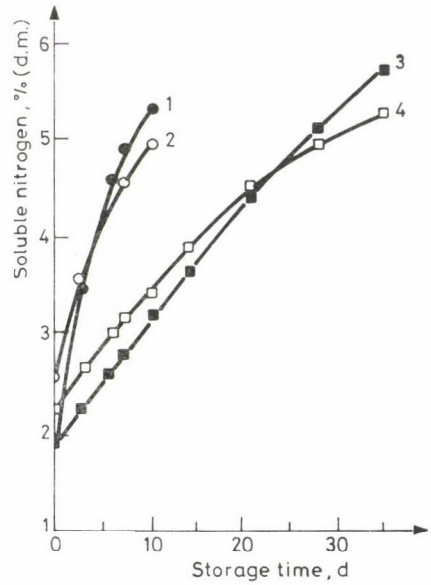


Fig. 3. Changes of soluble nitrogen content in Camembert and modified cheese during storage at 18 °C and 4 °C. ●——●: Camembert (18 °C); ○——○: modified (18 °C); ■——■: Camembert (4 °C); □——□: modified (4 °C)

life of Camembert cheese were obtained by KAMMERLEHNER and KESSLER (1982). They have found that Camembert cheese stored at 20 °C retained high quality during 5 days, at 15 °C during 10 days, and at 5 °C during 22 days. On the other hand, HERRMANN and co-workers (1980) found that by storing Camembert cheese at 2–4 °C its shelf life can be extended to 28–30 days from the date of its packing. It can be supposed that the higher stability of cheese manufactured according to the modified production process, compared to standard Camembert cheese, was due to the considerable decrease in moisture content as well as the fermentation material in the form of lactose.

Cheese manufacture according to the modified production process causes less intensive growth of the mould on its surface and consequently less inten-

Table 3

Parameters for the mathematical description of cheese quality factors changes during storage (model $y = ax^2 + bx + c$)

Sample	A				B			
Characteristics	value							
	a	b	c	R	a	b	c	R
Organoleptic evaluation[a] (points)	0.004	−0.344	5.07	0.992	−0.013	−0.119	4.99	0.999
Acidity (°SH)	0.299	−4.725	65.61	0.994	−0.150	−0.534	52.25	0.998
pH	−0.015	0.325	4.30	0.979	0.005	0.010	4.78	0.962
Soluble nitrogen (% d.m.)	−0.019	0.515	1.91	0.984	−0.015	0.398	2.55	0.987
Maturity degree	0.046	4.564	37.38	0.963	0.029	4.223	36.91	0.938
Free fatty acids (μEq/g)	0.050	2.293	9.77	0.958	0.030	2.331	9.63	0.963

Sample	C				D			
Characteristics	value							
	a	b	c	R	a	b	c	R
Organoleptic evaluation[a] (points)	−0.001	−0.028	4.88	0.998	−0.004	−0.039	5.01	0.999
Acidity (°SH)	0.037	−2.339	67.32	0.992	0.002	−0.770	52.00	0.941
pH	−0.002	0.128	4.42	0.997	−0.001	0.095	4.81	0.990
Soluble nitrogen (% d.m.)	−0.001	0.140	1.86	0.997	−0.001	0.140	2.26	0.932
Maturity degree	0.003	1.685	34.54	0.942	−0.006	1.776	37.71	0.955
Free fatty acids (μEq/g)	−0.023	0.662	17.96	0.946	−0.012	0.455	12.90	0.943

y: value of quality factor, x: storage time (days)
A: Camembert stored at 18 °C
B: modified cheese stored at 18 °C
C: Camembert stored at 4 °C
D: modified cheese stored at 4 °C

R: correlation coefficient
a, b, c: constants of equation
[a] critical characteristics

sive hydrolysis of protein by mould enzymes. Similar results were obtained by KAMMERLEHNER and KESSLER (1982). They achieved prolongation of Camembert cheese shelf life up to 24 days from the date of its packing by additional application in the production process of further drying of curd cubes, thus decreasing the moisture content by 6%. Also STEHLE (1980) is of the opinion that in stabilized cheese manufactured in France, considerable prolongation of cheese shelf life has been achieved when compared to standard Camembert due to flushing the cubes of cheese with water at a temperature of 35 °C, in quantity of 25–30% of the drained-off whey.

On studying changes in the quality of the products it was found that the change in quality characteristics as a function of time can be approximately described with quadratic equation. Procured correlation coefficient ($R > 0.937$) between actual and theoretical values can testify to this fact (Table 3).

The presentated mathematical formulae still do not form a full simple and, in practice, easy to use model. They enable only the prognosis of changes of product quality parameters during storage under conditions for which they have been determined. However, they represent a step towards the construction of more comprehensive and useful models.

3. Conclusions

3.1. Proper storage conditions

Assurance of proper storage conditions for Camembert cheese is decisive for its shelf life. Camembert cheese maintains proper qualities when stored at temperature of 4 °C during 21 days from the date of its packing while when stored at a temperature of 18 °C, about 3 days only.

3.2. Effect of modified cheese production process

Modification of the cheese production process applied in this investigation results in a considerable prolongation of shelf life. Stability of cheese manufactures according to the modified process, and stored at a temperature of 4 °C, is equal to 28 days. Stored at a temperature of 18 °C it is 6 days, and longer by 7 and 3 days, respectively, in comparison with the stability of standard Camembert cheese.

3.3. Critical quality factors of cheese

Critical quality factors of cheese with surface mould are the taste and the smell as well as the content of the soluble nitrogen compounds. Their critical values are 3 points on the 5 point scale of total organoleptic evaluation and content 4.5% of soluble nitrogen compounds in the dry matter of cheese.

Literature

BARYŁKO-PIKIELNA, K. (1975): *Zarys analizy sensorycznej żywności.* (Outline of sensory evaluation of food.) Wydawnictwa Naukowo-Techniczne, Warszawa, pp. 296–337.

BOULANGER, P. (1949): *Bull. Soc. Chem. Biol., 31,* 696–698.

BOZYK, Z. & RUDZKI, W. (1977): *Metody statystyczne w badaniu jakości produktów żywnosciowych i chemicznych.* (Statistical methods in food research and quality control.) Wydawnictwa Naukowo-Techniczne, Warszawa, pp. 103–138.

BUDSŁAWSKI, J. (1972): *Metody analizy żywności.* (Methods of food analysis.) Wydawnictwa Naukowo-Techniczne, Warszawa, pp. 228–238.

DEETH, U. C., FITZ-GERALD, G. H. & MADSEN, O. (1976): *Lipolysis in dairy products.* Research report. Dairy Research Laboratory. Department of Primary Industries Hamilton, Queensland.

HERRMANN, U., RUDOLF, B. & BURKHARDT, K. (1980): Zur befristeten Haltbarkeits-verlangerung von Weichkäse mit Schimmelbildung durch Kuhllagerung. *Milchforschung-Milchpraxis, 22,* 136–137.

KAMMERLEHNER, J. & KESSLER, H. G. (1982): Einflüsse auf die Haltbarkeit von Camembert. *Dte. Molk. Ztg., 103,* 1725–1728.

LABUZA, T. P. & RIBOH, E. (1982): Theory and application of Arrhenius kinetics to the prediction of nutrient losses in food. *Fd Technol., 36,* 66–74.

LENZ, M. K. & LUND, D. B. (1980): Experimental procedures for determining destruction kinetics of food components. *Fd Technol., 34,* 51–55.

MISHKIN, M., SAQUY, I. & KAREL, M. (1984): A dynamic test for kinetic model of chemical changes during processing: ascorbic acid degradation in dehydratation of potatoes. *J. Fd Sci., 49,* 1267–1270, 1274.

POZNAŃSKI, S. & SIUDAK, F. (1971): Wertości odżywcze białka wybranych typów serów podukowanych w Polsce. (Nutritive quality of protein of the chosen kind of Polish cheeses.) *Roczniki Chemii Techn. Zywn., 20,* 15–26.

POZNAŃSKI, S., BEDMARSKI, W., ŚMIETANA, Z. & CICHON, R. (1979): Perspektywy produkcji serów o wyższej jakości. (The prospects of the better quality cheeses production.) *Przeg. Mlecz., 2,* 19–22.

SCHNEIDER, K. & ROEDER, K. (1979): *Die praktische Milchprüfung und die Kontrolle von Molkereiprodukten.* Verlag K. J. Wyss, Erben AG, Bern.

STEHLE, G. (1980): Beiträg zur Technologie der Haltbarkeits-verlangerung von Weichkäse. *Verpackung Rundschau, 5,* 670–674.

ŚMIETANA, Z., POZNAŃSKI, E. & REPS, A. (1973): Witaminy występujące w serach. (Vitamins in cheeses.) *Przeg. Mlecz., 7,* 16–19.

Acta Alimentaria, Vol. 19 (4), pp. 331–336 (1990)

EFFECT OF RADIATION AND SOAKING ON PHYTATE CONTENT OF SOYBEAN

ABDUS SATTAR[a], NEELOFAR[b] and M. A. AKHTAR[b]

[a] Nuclear Institute for Food and Agriculture, Peshawar, Pakistan
[b] Department of Chemistry, University of Peshawar, Peshawar, Pakistan

(Received: 10 October 1989; accepted: 14 March 1990)

Biochemical composition of soybean was determined. Effect of irradiation (0.25–1.00 kGy) and soaking (3–12 hours) in tap and distilled water at ambient conditions and 50 °C on phytate content was studied. Phytate content significantly decreased with increasing soaking time and irradiation dose. The rate of phytate removal was generally greater during soaking at 50 °C than ambient temperature ($P < 0.01$). Loss of phytate was more on soaking in distilled than in tap water. Irradiation doses independently decreased the original phytate (212.0 mg per 100 g) to a range value of 182.5–102.5 mg per 100 g, while soaking in tap and distilled water for 12 hours to 78.9–109.5 mg per 100 g and 73.7–87.5 mg per 100 g, respectively. Combination treatment resulted in greater destruction of phytate than either one. Maximum destruction of phytate content (from 212.0 to 37.5 mg per 100 g) occurred on soaking at 50 °C of 1.0 kGy sample in distilled water.

Keywords: radiation and soaking treatment, phytate content of soybean, biochemical composition of soybean

Soybean has attracted much attention as a protein food and oil source in Pakistan in recent years and is being successfully used in child feeding programmes and other food and feed formulations in several countries. Phytic acid is a common constituent of plant tissues. The ability of phytate and its derivatives to complex and reduce the bioavailability of certain essential metals in diets containing plant proteins is a problem of concern to nutritionists (EL-MAHDY & EL-SEBAIY, 1982; MAGA, 1982). In addition to supplementation for improving nutritional value, soaking and germination of seeds have been suggested (KHAN & GHAFOOR, 1987). Soybeans are normally soaked prior to cooking in this and other countries. Although the influence of soaking on phytic acid in soybeans and other legumes, has been reported to be negligible (TABEKHIA & LUHN, 1980; EL-SHIMI et al., 1984), effect of radiation alone or in combination with soaking treatment has not been studied. However, recently SATTAR and co-workers (1989) observed higher loss of phytate during soaking of mungbean at 22–25 °C than at 55 °C. The US Food and Drug Administration (FDA) concluded in November, 1980 that "food irradiated to doses up to 1.0 kGy is wholesome and safe for human consumption and needs no safety tests to market the irradiated food" (ANONYMOUS, 1981). This paper reports the results of our investigation on the effect of radiation and soaking on phytic acid content of soybean.

1. Materials and methods

Soybean of commercial uniform variety (Woodworth), was obtained from the Nuclear Agriculture Division of this Institute. The seeds were dried under sunlight to a moisture content of about 10%.

1.1. Irradiation

The seeds were irradiated with gamma rays at a dose of 0.25, 0.50, 0.75 and 1.00 kGy in a Issledovatel (USSR) Irradiator with a dose rate of 9.6 kGy h^{-1}.

1.2. Soaking

Mature, unbroken seeds were soaked in 4–5 volumes of water (22–25 °C) for 3–12 h at room temperature (25–35 °C) and 50 °C under prevailing light conditions in the laboratory in tap and distilled water in each case.

1.3. Biochemical analysis

The soaked seeds were taken every 3 hours, dried at about 60 °C in an air oven, ground in a Wiley mill to pass through a 40 mesh sieve and stored in plastic bottles in a deep freezer for subsequent analyses. Moisture was determined by drying at 105 °C. Proximate analysis was performed in accordance with A.O.A.C. (1984) procedures. Crude fat was determined by Soxhlet extraction using petroleum ether (b.p. 40–60 °C) with the help of Tecator Soxtec system model 1043—44. Crude protein (% N×6.25) was determined by the micro-Kjeldahl method using Vapodest system, mineral matter (ash) by heating at 550 °C and crude fibre by Tecator system model 1020—2122. The food energy was calculated from the proximate analysis data by multiplying the fat by 9.0, and protein and carbohydrates by 16.74 kJ g^{-1}. Assay for phytate content were done according to the method of WHEELER and FERREL (1971). Phytate was extracted with trichloroacetic acid as the ferric salt. The iron contents were measured by spectrophotometry at 480 nm using Shimadzu (model UV-160) UV-visible recording spectrophotometer. The results were used to calculate the phytate phosphorus content of samples assuming a constant 4Fe : 6P molecular ratio in the precipitate. The results were presented as the means of duplicate determinations. The relative standard deviation on the consecutive determination in the same sample was 3–5% (SD range 2.12–4.05).

1.4. Statistical analysis

Statistical significance of treatments was tested by the two-way analysis of variance and the means were compared using LSD at 1% probability level (LITTLE & HILLS, 1972).

2. Results and discussion

The analysis of soybean for proximate composition and some vitamins and essential metals showed that this legume contained moisture 8.2%, mineral matter 4.5%, protein 42.3%, fat 14.8%, fibre 5.2%, and carbohydrates 25.0% with an energy value of 1548.6 kJ per 100 g. The concentration of trace elements was iron 160.00, Cu 12.16, Mn 37.40, and Zn 40.60 μg per g. Ascorbic acid was not deteetable and the riboflavin was 3.30 μg per g. The results are generally similar to the values reported earlier (REDDY et al., 1982; GANDHI et al., 1985; ZEB et al., 1987). Lack of ascorbic acid in the legume is not surprising because food grains are not considered a source of this vitamin. Presence of considerable amount of phytate would certainly limit the usefulness of this food legume.

The data on phytate as a results of irradiation and subsequent soaking in tap and distilled water at ambient conditions are presented in Table 1. The phytate content exhibited a decreasing pattern as a result of soaking, irradiation and combination treatments. Removal of phytate was higher during soaking in distilled than tap water. Soaking of unirradiated sample for 12 hours decreased this biochemical factor from 212.0 to 109.5 and 78.9 mg per 100 g (48% and 63% loss) in tap and distilled water, respectively. Radiation treatments alone (0.25–1.00 kGy) lowered the phytate content to a range value of

Table 1

Effect of irradiation and soaking at ambient conditions on phytate content of soybean
(mg per 100 g)[a]

Soaking time (h)	Unirradiated (control)	Radiation doses (kGy)				Mean
		0.25	0.50	0.75	1.00	
0	212.0	182.5	135.0	110.0	102.0	148.3
3	171.3 (145.0)	113.5 (140.0)	108.0 (119.0)	102.5 (109.5)	93.5 (97.5)	117.7 (122.2)
6	139.0 (100.0)	107.5 (90.0)	101.5 (85.0)	91.0 (83.0)	71.5 (72.5)	101.1 (86.1)
9	121.7 (90.0)	100.5 (83.0)	86.2 (79.7)	81.0 (70.2)	64.5 (63.2)	90.7 (77.2)
12	109.5 (78.9)	81.0 (76.7)	77.5 (70.0)	61.9 (60.5)	60.0 (56.5)	78.0 (68.5)
Mean	150.7 (125.2)	117.0 (114.4)	101.6 (97.7)	89.3 (86.6)	78.2 (78.3)	

[a] Soaking in tap water. Values in parenthesis represent soaking in distilled water.
LSD 1% — Radiation doses/soaking time = tap-water 2.66; distilled water 3.05
SE — Radiation doses/soaking time = tap-water 0.70; distilled water 0.81
SE — Radiation doses × soaking time = tap-water 1.57; distilled water 1.80

Table 2

Effect of irradiation and soaking at 50 °C on phytate content of soybean (mg per 100 g)[a]

Soaking time (h)	Unirradiated (control)	Irradiated doses (kGy)				Mean
		0.25	0.50	0.75	1.00	
0	212.0	182.5	135.0	110.0	102.5	148.4
3	135.0 (108.2)	127.5 (102.5)	120.0 (91.4)	107.5 (86.0)	97.5 (76.0)	117.5 (92.8)
6	125.0 (90.6)	120.0 (82.5)	112.0 (67.5)	105.0 (60.0)	92.5 (57.5)	110.9 (71.5)
9	107.5 (88.6)	100.0 (75.5)	95.0 (58.5)	90.0 (52.9)	85.0 (47.5)	95.5 (64.5)
12	87.5 (73.7)	82.5 (64.37)	80.0 (53.5)	75.0 (47.0)	70.0 (37.5)	79.0 (65.2)
Mean	133.4 (114.6)	122.5 (101.4)	108.4 (81.2)	97.5 (71.2)	89.5 (64.0)	

[a] Soaking in tap water. Values in parenthesis represent soaking in distilled water
LSD 1% — Radiation doses/soaking time = tap-water 2.31; distilled water 1.03
SE — Radiation doses/soaking time = tap-water 0.61; distilled water 0.27
SE — Radiation doses × soaking time = tap-water 1.36; distilled water 0.61

182.5–102.0 mg per 100 g (14–52% loss). Combination of soaking and irradiation resulted in greater elimination than either treatment alone. The decreases to values of 60.0 and 56.5 mg per 100 g occurred during soaking at ambient conditions for 12 hours of 1.0 kGy sample (71.7 to 73.3% loss) using tap and distilled water, respectively. In an earlier study (ZEB et al., 1987), it was observed that destruction of phytate was higher during soaking of soybean at 50 °C than ambient conditions, it was therefore considered worthwhile to determine the influence of irradiation and soaking at 50 °C using both tap and distilled water. The results of this experiment are shown in Table 2. Under these conditions, soaking of untreated soybeans decreased the phytate level to 87.5 and 73.7 mg per 100 g (59 and 65% loss) in tap and distilled water, respectively. Maximum decreases to values of 70.0 and 37.5 mg per 100 g from initial amount of 212.0 mg per 100 g occurred on soaking at 50 °C for 12 hours of 1.0 kGy treated sample (67 and 82% loss) in tap and distilled water, respectively. The difference between tap water and distilled water is significant only in case of control but not in those of irradiated samples at room temperature. At 50 °C the difference between the soaking agents remains significant as a function of radiation dose and soaking time. Statistical analysis of the data by the analysis of variance clearly indicated that there were significant variations ($P \leq 0.01$) in the phytate content in relation to treatments such as radiation doses and soaking times. The combined influence of treatments was also significant ($P \leq 0.01$). The

LSD values ($P \leq 0.01$) have been provided for each treatment and their interactions alongwith the standard errors (SE) for comparison of the data values and their means.

Although removal of phytate in relation to soaking and germination has been observed (SATTAR et al., 1985; 1989) significant contribution of irradiation alone or in combination with soaking has not been reported in the literature. Faster degradation of phytate in distilled than tap water is attributed to the absence of possibly metal–phytate complex thus facilitating diffusion of phytic acid from the soaked seeds. Moreover the distilled water improved the phytate removal and/or hydrolysis compared to the water containing different salts (Iyer et al., 1980 cited by REDDY et al., 1982). The same pattern recently has indeed been observed in germinating soybeans (SATTAR et al., 1990). Higher degradation of phytate during soaking at 50 °C than 22–25 °C especially in distilled water is attributed to the combined effects of phytase and diffusion. However, the reason for the absence of similar pattern during soaking of irradiated sample in tap water is not clear. EL-MAHDY and EL-SEBAIY (1982) indeed reported greater activity of phytase and phosphatase at 52 °C than 32 °C throughout soaking of Fenugreek seeds. An increase in the phytase activity with decrease in phytate, as a result of soaking and germination of Fababean, has also been observed (ESKIN & WIEBE, 1983). In addition to the enzymatic hydrolysis of phytate, CHANG and co-workers (1977) proposed diffusion to be an important process for the removal of phytate in dry beans. Extraction pattern of phytin-P in green gram revealed higher extraction in 5*N* HCl than water (KUMAR et al., 1978). However, in the case of beans, all the phytin-P was found to be water soluble (LOLAS & MARKAKIS, 1977). This indicated that the removal of phytate during soaking depends upon the nature of phytin, which may be in the form of K, Ca or Mg salts.

It was concluded that soaked soybeans contain significantly lower amount of phytic acid than unsoaked originals. Irradiation of seeds alone or in combination with soaking results even greater destruction of phytate. Even though pH-dependent solubilization and prolonged heat treatments could eliminate most of the phytic acid in legumes, excessive treatments could cause functional as well as nutritional damage to protein. In order to avoid these damaging effects, soaking or germination following low dose irradiation might well be a safe process for eliminating phytic acid problem.

*

We are thankful to Dr. I. KHAN, Director for general assistance and Mr. S. K. DURRANI and Mr. F. MAHMOOD for technical help during this study.

References

ANONYMOUS (1981): Policy for irradiation: Advances notices of preparing procedures for the regulation of irradiated foods for human consumption. *Fed. Register*, USA *46(59)*, 18992.

A. O. A. C. (1984): Official methods of analysis. 14th ed. Association of Official Analytical Chemists, Washington D. C.

CHANG, R., SCHWIMMER, S. & BURR, H. K. (1977): Phytate: Removal from whole dry beans by enzymatic hydrolysis and diffusion. *J. Fd Sci., 42*, 1098–1101.

EL-MAHDY, A. R. & EL-SEBAIY, L. A. (1982): Changes in phytate and minerals during germination and cooking of Fenugreek seeds. *Fd Chem., 9*, 149–158.

EL-SHIMI, N. M., DAMIR, A. A. & RAGAB, M. (1984): Changes in some nutrients of Fenugreek seeds during germination. *Fd Chem.*, 14, 11–19.

ESKIN, N. A. M. & WIEBE, S. (1983): Changes in phytase activity and phytate during germination of two Fababean cultivars. *J. Fd Sci., 48*, 270–271.

GANDHI, A. P., MENWANI, M. M. & ALI, N. (1985): Some physico-chemical characteristics of soybeans (*Glycine max*, Merr). *Fd Chem., 17*, 71–74.

KHAN, M. A. & GHAFOOR, A. (1987): The effect of soaking, germination and cooking on the protein quality of mash beans *(Phaseolus mungo)*. *J. Sci. Fd Agric., 29*, 461–464.

KUMAR, K. G., VANKATARAMAN, L. V., JAYA, T. V. & KRISHNAMURTHY, K. S. (1978): Cooking characteristics of some germinated legumes; changes in phytins, Ca^{++} and pectins. *J. Fd Sci., 43*, 85–88.

LITTLE, J. M. & HILLS, F. J. (1972): Statistical methods in agriculture research. Agric. Extns. Univ. Calif., USA. pp. 50–51.

LOLAS, G. M. & MARKAKIS, P. (1977): Phytic acid and other phosphorus compounds of beans *(Phaseulus vulgaris L.) J. agric. Fd Chem., 23*, 13–15.

MAGA, J. A. (1982): Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *J. agric. Fd Chem., 30*, 1–9.

REDDY, N. R., SATHE, S. K. & SALUNKHE, D. K. (1982): Phytates in legumes and cereals. *Adv. Fd Res., 28*, 1–92.

SATTAR, A., NEELOFAR & AKHTAR, M. A. (1990): Irradiation and germination effects on phytate, protein and amino acids of soybean. *Plant Fd Hum. Nutr., 40*, 185–194.

SATTAR, A., DURRANI, S. K. MAHMOOD, F., AHMAD, A. & KHAN, I. (1989): Effect of soaking and germination temperatures on selected nutrients and anti nutrients of mungbean. *Fd Chem., 34*, 111–120.

SATTAR, A., MAHMOOD, F., KHAN, S., NEELOFAR & KHAN, I. (1985): Effect of irradiation and germination on selected nutrients of corn. *Fd Chem., 17*, 183–192.

TABEKHIA, M. M. & LUHN, B. S. (1980): Effect of germination, cooking and canning on phosphorus and phytate retention in dry beans. *J. Fd Sci., 45*, 406–408.

WHEELER, E. L. & FERREL, R. E. (1971): A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem., 48*, 312–320.

ZEB, A., SATTAR, A. & MOHAMMAD, J. (1987): Effect of soaking and heating on selected nutrients and phytate content of soybean and mungbean. *Sarhad J. Agric., 3*, 179–184.

Acta Alimentaria, Vol. 19 (4), pp. 337–346 (1990)

WATER VAPOUR SORPTION HYSTERESIS AND THE SHELF LIFE OF INDUSTRIAL SPONGE-CAKE

P. GUINOT[a] and M. MATHLOUTHI[b]

[a] Département de Biologie Appliquée, Institut Universitaire de Technologie Université de Nancy I, Le Montet, 54600 Villers-les-Nancy. France
[b] Laboratoire de Chimie Physique Industrielle, Faculté des Sciences, Université de Reims Champagne-Ardenne, B. P. 347, 51062 Reims Cédex. France

(Received: 29 November 1989; accepted: 5 March 1990)

Water vapour adsorption and desorption isotherms were determined at +4 and +20 °C for the ingredients constituting sponge-cake and water activity depressors (alignates and soy proteins). The effect of these additives, temperature and modified atmosphere (50% CO_2–50% N_2) preservation, on sorption hysteresis was investigated. The additive which contributes to hysteresis stabilization is soy proteins at a level of 1%. Sponge-cake is itself a raw material for number of preparations and it is important to increase its shelf-life.

Keywords: sponge-cake, water activity (a_w) depressors, sorption hysteresis, CO_2 preservation

The quality and shelf-life of intermediate moisture foods (I. M. F.) like sponge-cake depend essentially on the stability of their moisture. Indeed most of the quality characters like a smooth texture, the absence of microbial contamination or loss of weight are related to water content and water activity (a_w) of the product. The influence of water activity on the quality and stability of foodstuffs was discussed at a recent symposium (ROCKLAND & STEWART, 1981). It was shown (ACOTT & LABUZA, 1975) that for an intermediate moisture food prepared at a given a_w the stability increases when the moisture content decreases. An increase in water content may occur in a food product which is subject to water vapour sorption hysteresis and results in microbial spoilage. Depending on the way (adsorption or desorption) of obtaining the a_w value, the product is or is not microbiologically stable. If water activity is lowered by adding humectants, the stability is not always insured even if the a_w value is situated in a region where theoretically no microbial development should occur. Indeed, if glycerol or fructose are used as humectants, bacterial growth may be observed (SPERBER, 1983) because of the relatively high mobility of water molecules around these small hydrophilic substances (MATHLOUTHI, 1986). Other factors of alteration of preserved foods like non-enzymatic browning and lipid oxidation are sensitive to a_w. I. M. F. with a_w values (0.65–0.9) which are not situated in the optimal region of lipid oxidation are more sensitive to this

oxidation when they are prepared by water vapour adsorption than when they are prepared by desorption (LABUZA et al., 1972). Different examples may be cited to show that the composition, the technology of elaboration and the conditions (temperature, humidity) of packaging and storing of a foodstuff affect the retention of water and the stability. The phenomenon of water vapour sorption hysteresis seems to be a critical parameter for a product like industrial sponge-cake ($a_w = 0.91$). This is the reason why we investigated the modification of hysteresis as a function of formulation, storage temperature and composition of the modified atmosphere used for the preservation of the product.

1. Materials and methods

The investigated sponge-cake was prepared from an industrial mixture composed of half wheat flour, half sucrose and small amounts of baking powder and E471 and E472 emulsifiers. For 800 g of cake, 400 g of the mixture are mixed to 320 g of reconstituted egg (240 g water + 80 g egg powder) and 80 cm^3 of water at 45 °C. After mixing the ingredients, the cake was baked for 20 min at 180 °C temperature. The preparation of the reference sample is the same as previously described (GUINOT et al., 1988). In this study of water vapour sorption, two water activity depressors were used: soy proteins isolate (Soyamin® 90) offered by Lucas Meyer S. A., Le Blanc Mesnil, France, and sodium alginate (Sobalg® FD 155) supplied by Grinsted France.

The packaging film was supplied by Rhone-Poulenc Films. It is a high barrier complex with reinforced impermeability composed of metallized PET and polyolefins. Its trade name is Clarylene® HB. The permeabilities of Clarylene® HB to water vapour, O_2 and CO_2 are, respectively: < 0.2 g m^{-2} 24 h^{-1} (38 °C, Δ E. R. H. = 90%); < 0.02 cm^3 m^{-2} 24 h^{-1} (21 °C, 101 325 Pa) and 1 cm^3 m^{-2} 24 h^{-1} (21 °C, 101 325 Pa).

The samples of sponge-cake are obtained by cutting 2 mm thick lamellas of approximately 1 g weight inside the cake in order to prevent heterogeneity of moisture due to the crust. These samples are put in the bottom of vials which can be closed during the wheighing period. These vials are placed into tight elosing jars where we have previously distributed saturated salt solutions to adjust water activity ($LiCl \cdot H_2O$, $a_w = 0.12$; CH_3COOK, $a_w = 0.23$; $MgCl_2 \cdot 6H_2O$, $a_w = 0.33$; $Mg(NO_3)_2$ $a_w = 0.54$ NaCl, $a_w = 0.75$; $BaCl_2$, $a_w = 0.90$ and $K_2Cr_2O_7$, $a_w = 0.98$).

The water content of the product (33%) before sorption is determined by drying for 3 h at 105 °C in the oven. The evolution of the weight of the sample is followed until the equilibrium is reached after $\simeq$ 120 h. Each curve of sorption or desorption is repeated 4 times. No statistical calculation was performed as we observed that most of water content values for a given a_w are identical.

This was obtained by a suitable conditioning of air in the laboratory: 20 °C (± 1 °C) and the cold room: 4 °C (± 0.5 °C) where the gravimetric method (MATHLOUTHI et al., 1980) of sorption curve determination was performed.

2. Results and discussion

2.1. Sorption isotherms of raw materials

The isoterms of adsorption and desorption of water vapour by the flour mixture (wheat flour + sugar + baking powder + emulsifiers E471 and E472), the egg powder, soy proteins (Soyamin® 90) and sodium alginates were determined at 4 °C and 20 °C (± 1 °C). The results are shown in Figs. 1, 2, 3 and 4. Moisture in percentage of dry substance (% H_2O) is plotted as a function of a_w. The general aspect of the curves is that usually observed for foodstuffs

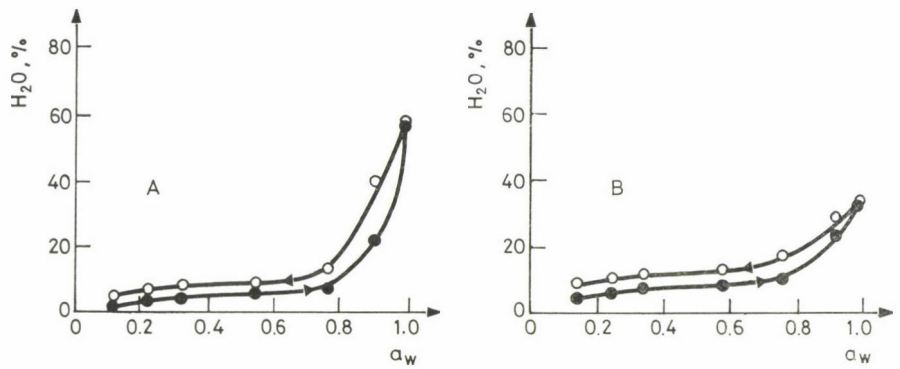


Fig. 1. Water vapour adsorption (●) and desorption (○) isotherms for the wheat flour mixture at 20 °C (A) and 4 °C (B)

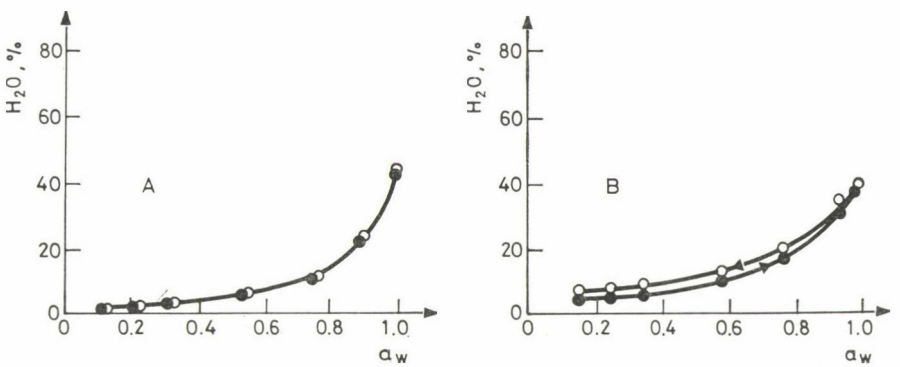


Fig. 2. Water vapour adsorption (●) and desorption (○) isotherms for the egg powder at 20 °C (A) and 4 °C (B)

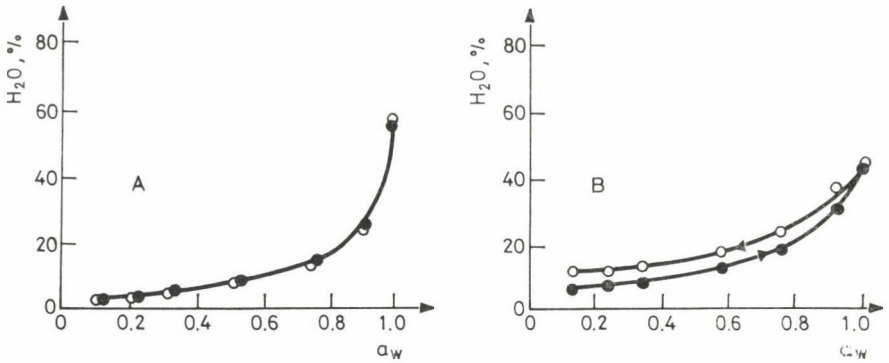


Fig. 3. Water vapour adsorption (●) and desorption (○) isotherms for soy proteins at 20 °C (A) and 4 °C (B)

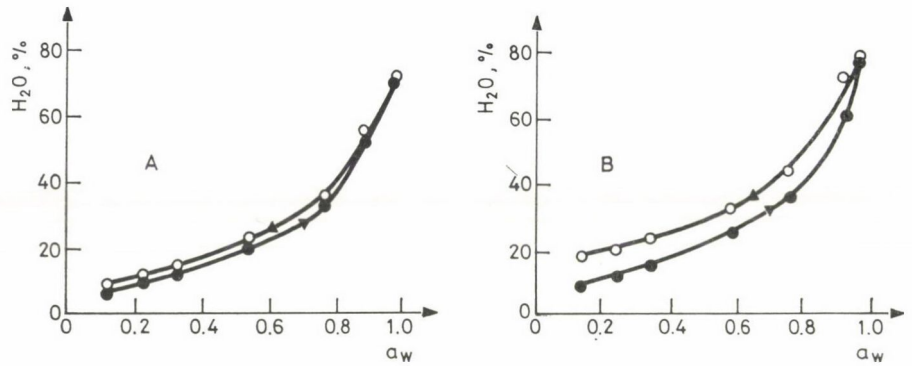


Fig. 4. Water vapour adsorption (●) and desorption (○) isotherms for sodium alginate at 20 °C (A) and 4 °C (B)

(Troller & Christian, 1978). It should be noted that egg powder and soy proteins do not exhibit any hysteresis between the adsorption and desorption curves at 20 °C (see Figs 2A and 3A). This phenomenon is observed at 4 °C for all products and at 20 °C for flour mixture and sodium alginates. It is not easy to interpret the sorption hysteresis. One of the explanations consists in recalling that water vapour adsorption or filling of capillaries needs a higher partial pressure than their desorption or emptying (Eichner, 1986). Kinetics of desorption is lower than that of adsorption. Moreover, the differences in sorption behaviour observed in Figs. 1–4 is related to water-substance interactions. The absence of hysteresis at 20 °C for soy proteins and egg powder is probably due to the fact that no capillaries are present in the samples and the strength of H bonds is the same during the adsorption and desorption stages. The effect of temperature, namely the presence of sorption hysteresis in all experimental curves at 4 °C, is very likely related to the physical properties of water itself. Indeed, it is well known that pure water shows a maximum of density at

+4 °C corresponding to shorter or more energetic H-bonds. The energy necessary to rupture such bonds and release water molecules in the vapour phase (desorption) is higher at 4 °C than it is at 20 °C. The results obtained with raw materials may help in interpreting the sorption behaviour of the baked goods. Modifications of temperature and relative humidity (RH) may occur during the storage of the product and result in sorption or desorption of water vapour. Our objective is to reach the stability of moisture content at a given temperature and RH. This may be obtained by adding soy proteins which are stable at 20 °C (see Fig. 2A). Minimization of water vapour sorption hysteresis for the sponge-cake should lead to increased shelf-life.

2.2 Adsorption and desorption of water vapour by sponge-cake

A tentative interpretation of water vapour sorption by raw materials was made. Other arguments may be given such as the modification of interfacial properties of adsorbed water (MATHLOUTHI, 1986). The presence of ten-

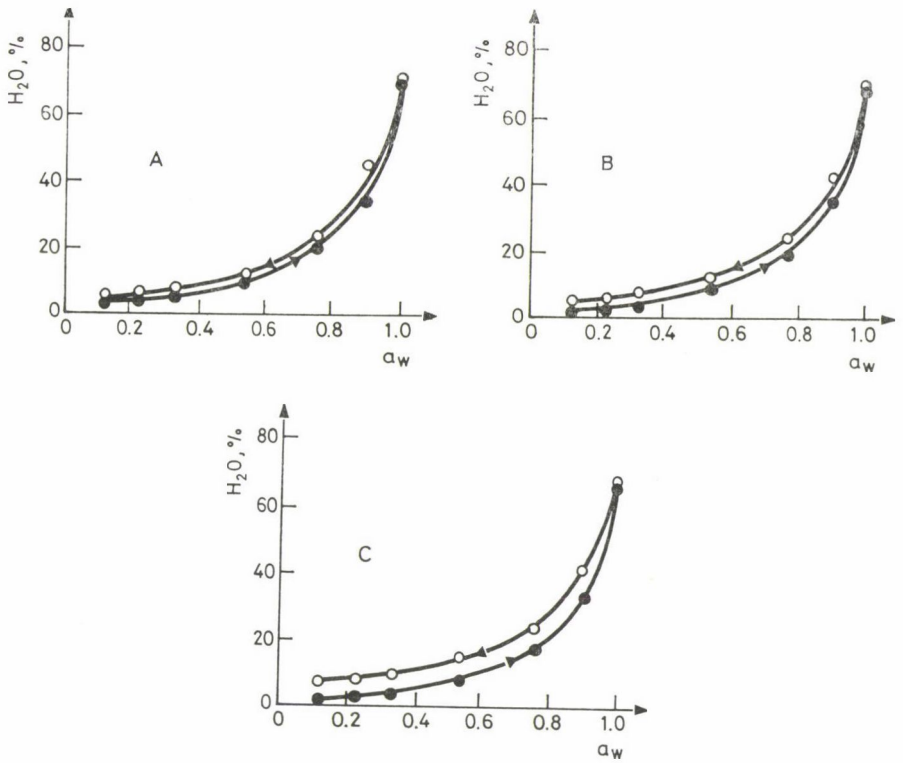


Fig. 5. Water vapour adsorption (●) and desorption (○) isotherms at 20 °C for a control sponge-cake (A), a 0.2% sodium alginate added sponge-cake (B) and a 0.5% sodium alginate added sponge-cake (C), after a 4 weeks storage under the air at 20 °C

sion-active agents may affect the sorption of water. Lecithin which is found in egg powder acts as a surface tension depressor on water and contributes to minimize sorption hysteresis. Among the parameters influencing water sorption by sponge-cake, the effect of additives (alginates and soy proteins), temperature and composition of the modified gas used for the preservation of the packed cake are studied.

2.2.1. Effect of alginates concentration. The rates of alginates added to the classical formula of sponge-cake are 0.2 and 0.5%. Sorption and desorption isotherms determined at 20 °C for reference sample (without additive) and samples with alginates are reported in Fig. 5. Observation of these results shows an increase of the hysteresis of water vapour sorption with the concentration of alginates. The behaviour of alginates is comparable to that of other charged polysaccharides like pectins. For these macromolecules (pectins) the adsorption of water produces structural modifications of the chains of polygalacturonic acid which are observed on X-ray diffraction patterns (BETTELHEIM et al., 1956). Intermolecular distances increase with relative humidity in a way similar

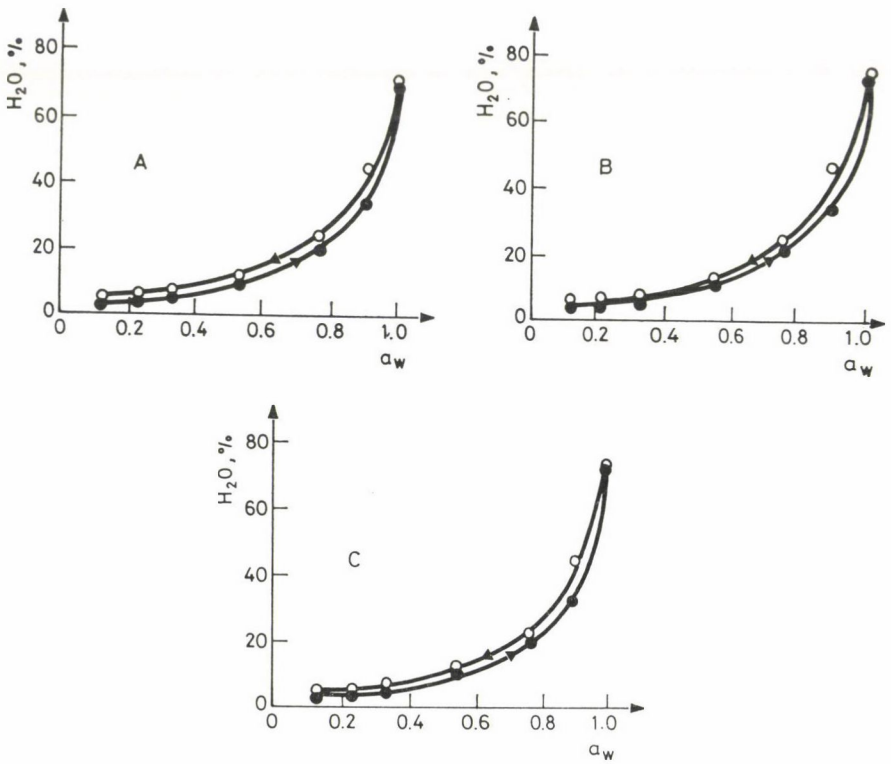


Fig. 6. Water vapour adsorption (●) and desorption (○) isotherms at 20 °C for a control sponge-cake (A), a 0.5% soy proteins added sponge-cake (B) and a 1.0% soy proteins added sponge-cake (C), after a 4 weeks storage under the air at 20 °C

to the general aspect of water vapour sorption curve. Adsorption and desorption curves describe pseudo-equilibrium states. It is especially the case for desorption of water from alginates which is a slow mechanism at the origin of the hysteresis phenomenon (see Figs. 5B and 5C).

2.2.2. Effect of soy proteins concentration. The rates of soy proteins added to the formula of sponge-cake are 0.5 and 1%. Water vapour sorption isotherms determined at 20 °C are reported in Fig. 6 for the reference sample and cake with soy proteins. No significant difference in the behaviour of cakes with and without additive is observed. This result is in agreement with the sorption isotherm at 20 °C for pure soy proteins (see Fig. 3A). Indeed no hysteresis is observed in Fig. 3A. Addition of soy proteins contributes to the decrease of surface tension of water from 72 mN m^{-1} to about 30–40 mN m^{-1}. This decrease is due to the well-known emulsifying effect of soy proteins. The differences in contact angles of water with the product are minimized between adsorption and desorption steps. This leads to easier filling and emptying of the capillaries and leads to a minimized hysteresis as observed in Fig. 6.

2.2.3. Effect of temperature. The effects of additives were investigated at room temperature ($\simeq$ 20 °C) for two concentrations. Another temperature of

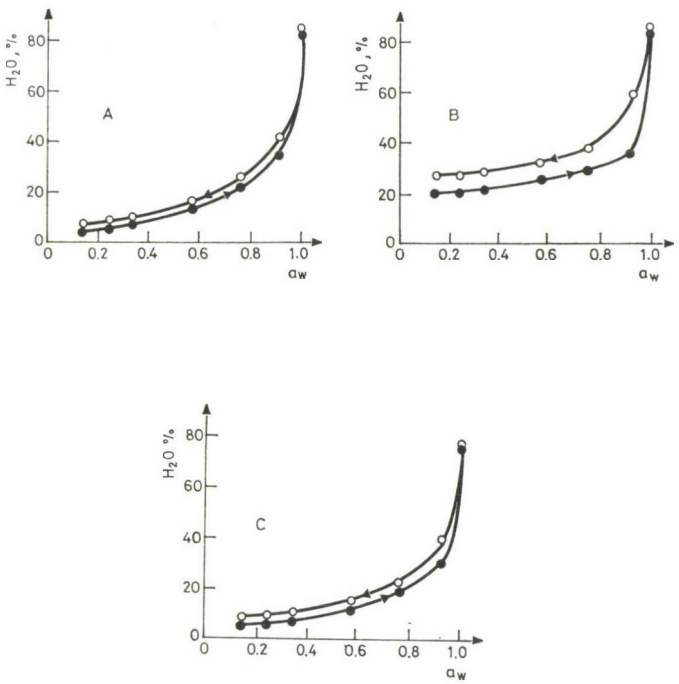


Fig. 7. Water vapour adsorption (●) and desorption (○) isotherms at 4 °C for a control sponge-cake (A), a 0.5% sodium alginate added sponge-cake (B) and a 1% soy proteins added sponge-cake (C), after a 4 weeks storage under the air at 4 °C

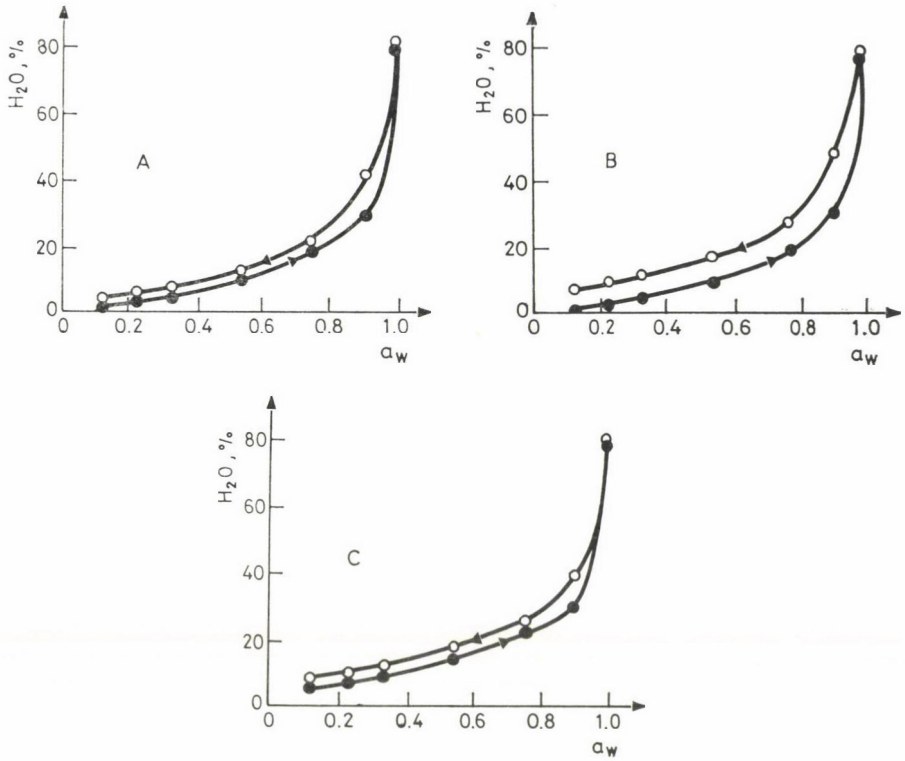


Fig. 8. Water vapour adsorption (●) and desorption (○) isotherms at 20 °C for sponge-cake (without additive) (A), with 0.5% sodium alginate (B); and with 1% soy proteins (C); after a 4 weeks storage under the modified atmosphere (50% CO_2 – 50% N_2) at 20 °C

storage is interesting to investigate. It is the temperature of refrigerated storage at 4 °C. Water vapour sorption isotherms were determined at 4 °C for a test sample and two samples with 0.5% alginates and 1% soy proteins, respectively. The results are shown in Fig. 7. Comparison of Fig. 7 with Fig. 5 and Fig. 6 reveals the effect of temperature lowering on sorption hysteresis. No noticeable modification is observed for the test sample (without additive). The sorption curves for the samples prepared with 1% soy proteins are comparable at 20 and 4 °C (see Figs 6C and 7C). Observation of Figs. 5C and 7B shows an increase in hysteresis when temperature is lowered from 20 to 4 °C. Moreover, moisture content is 3 to 4 times higher at 4 °C as compared to 20 °C. The change in behaviour of sponge-cake with alginates is attributable to the change in the structure of alginates with temperature and humidity. The swelling of these polysaccharides seems to be enhanced when the temperature is lowered.

2.2.4. Effect of modified atmosphere packaging. Water vapour adsorption and desorption isotherms (at 20 °C) were determined for a reference sample, and

cakes with 0.5% alginates or 1% soy proteins after 4 weeks preservation in a modified atmosphere composed of 50% CO_2 and 50% N_2. The results are reported in Fig. 8. Comparison of sorption curves of Fig. 8 with Figs. 5 and 6 permits evidencing of the effect of a modified atmosphere on water sorption hysteresis. Sponge-cakes without additives or supplemented with 1% soy proteins show comparable difference between adsorption and desorption of water vapour, whereas the variation between Figs. 5C and 8B is observable for $a_w = 0.8$. For these values an increase in water retention and hysteresis is observed for the sample with alginates submitted to modified atmosphere packaging. The curves obtained after 10 weeks of preservation in 50% CO_2 – 50% N_2 atmosphere are identical to what is observed in Fig. 8 and are not reported. We have previously (GUINOT et al., 1988) observed that CO_2 has no significant influence on a_w of sponge-cake. It was also demonstrated (GUINOT, 1988) that CO_2 is not adsorbed on the ingredients composing the cake. It is normal then that modified atmosphere packaging does not influence the sorption hysteresis of sponge-cake.

3. Conclusion

In order to stabilize an I. M. F. like sponge-cake with $a_w = 0.91$ during its preservation, one of the means consists in minimizing the water vapour sorption hysteresis, and to lower a_w. Addition of water activity depressors of different origins (proteins and charged polysaccharides) was studied. The effect of polysaccharides (sodium alginates) was found opposed to the desired objective. These additives contribute to increase sorption hysteresis especially at lower temperatures (4 °C). When soy proteins are added at a level of 1%, the product is stabilized. The sorption hysteresis does not increase or decreases slightly at 20 °C. No significant effect of modified atmosphere (50% CO_2 – 50% N_2) packaging is observed on water vapour sorption hysteresis. Sponge-cake which may be used as a raw material for number of pastry preparations may be prepared with 1% of soy proteins and preserved under a 50% CO_2 – 50% N_2 atmosphere in a gastight plastic package during 10 weeks at room temperature (20 °C) without any microbial spoilage or perceived change in organoleptic properties (GUINOT, 1988). Our approach of the optimized conditions for the preservation of sponge-cake permitted definition of the optimal mixture of gas (50% CO_2 – 50% N_2) and the optimal packaging film (Clarylene® HB) in the first paper (GUINOT et al., 1988) and the optimal dose of additive and temperature in this study. All the studied parameters affect in a way or another water availability for spoilage reactions.

*

The authors thank Mr. J. P. DE LEIRIS (Rhone-Poulenc Films) for his interest in this work and for supplying the film. Our thanks are extended to the Society SIAC (Airgaz), Lucas Meyer S. A. and Grinsted France for free samples.

References

ACOTT, K. M. & LABUZA, T. P. (1975): Microbial growth response to water sorption preparation. *J. Fd Technol., 10,* 603–611.

BETTELHEIM, F. A., STERLING, C. & VOLMAN, D. H. (1956): Pectic substances water. — Part I. Structural changes in the polygalacturonide chains during water adsorption. *J. Polym. Sci., 22,* 303–314.

EICHNER, K. (1986): The influence of water content and water activity on chemical changes in foods of low moisture content under packaging aspects. — in: MATHLOUTHI, M. (Ed.) *Food packaging and preservation. Theory and practice.* Elsevier Applied Science Publishers, London, pp. 67–92.

GUINOT, P. (1988): *Contribution à l'étude de la prolongation de la durée de vie d'une génoise industrielle emballée sous un mélange de gaz carbonique et d'azote.* Thesis, University of Dijon, pp. 81–148.

GUINOT, P., MATHLOUTHI, M. & DE LEIRIS, J. P. (1988): Effect of low residual O_2 and CO_2 on the preservation of CO_2- and N_2-packed sponge-cake. *Acta Alimentaria, 17,* 183–192.

LABUZA, T. P., MCNALLY, L., GALLAGHER, D., HAWKES, J. & HURTADO, F. (1972): Stability of intermediate moisture foods. — Part I. Lipid oxidation. *J. Fd Sci., 37,* 154–159.

MATHLOUTHI, M. (1986): Water interactions and food preservation. — In: MATHLOUTHI, M. (Ed.) *Food packaging and preservation. Theory and practice.* Elsevier Applied Science Publishers, London, pp. 137–164.

MATHLOUTHI, M., CONRY, M., JAILLANT, G. & MAITENAZ, P. C. (1980): Water vapour sorption of gruyere cheese. *Lebensm. Wiss. Technol., 13,* 264–268.

ROCKLAND, L. B. & STEWART, G. F. (1981): *Water activity: Influences on food quality.* Academic Press, New York.

SPERBER, W. H. (1983): Influence of water activity on foodborne bacteria. A review. *J. Fd Prot., 46,* 142–150.

TROLLER, J. A. & CHRISTIAN, J. H. B. (1978): *Water activity and food.* Academic Press, New York, pp. 5–9.

Acta Alimentaria, Vol. 19 (4), pp. 347–357 (1990)

VARIETAL AND CHEMICAL ASPECT OF TOMATO PROCESSING

H. G. DAOOD, M. A. AL-QITT, K. A. BSHENAH and M. BOURAGBA

Higher Institute of Technology, P. O. Box 68, Brack. Libya

(Received: 16 January 1990; accepted: 18 April 1990)

A comparative study was undertaken to investigate the quality parameters of the three most familiar tomato cultivars (American Rabb, Italian Roma VF and local Arab) cultivated in the area of the Wadi Ashati of Libya. The results showed that, apart from the large size of the fruits, the American cultivar "Rabb" gives a product with the best technological properties such as total soluble solids (TSS), titrable acidity, pectin and carotenoid content. The different cultivars were found to vary substantially in the above-mentioned parameters as well as in the others such as total solids (TS), reducing sugar, vitamin C and iron content of the fruits.

The effects of processing on the quality attributes of tomato products were also studied. While the TSS content of the juice was not influenced by mechanical extraction, ascorbic acid and carotenoid pigments were destroyed to a high extent. In contrast the iron content of the dry matter increased after such extraction. Depending on the temperature and absence or presence of molecular oxygen, there was a remarkable variation among heat treatments in their effects on the quality components of processed tomato products such as juice and paste.

Keywords: tomato, quality parameters, processing

Tomato *(Lycopersicon esculentum)* is one of the most important vegetable crops being consumed and/or processed world-wide. It is an important fact that to obtain a high quality product, the use of tomato fruit of good technological characteristics is very necessary. Inheritance of tomato fruit quality components such as soluble solids, acidity, colour, flavour, etc. has been published in the survey of STEVENS (1986). Such quality components are strongly associated with the fruit composition (FARKAS, 1987).

In Libya, nowadays an increasing attention is being paid to vegetable production and processing in the areas of oasis where the water supply is available at a low cost. Wadi Ashati is one of these areas. However outstanding are the characters of the varieties being adapted in such areas, the yield and quality components of vegetable crops may positively or negatively respond to the new environmental factors, such as climate, weather, sunshine period, etc. (AUNG, 1979). Therefore it seems to be of special importance to check such parameters in any vegetable crop to ascertain its suitability for processing when produced under new environmental conditions.

The object of this study was to get comparative data on the quality components of the three most important varieties of tomato produced in Wadi Ashati area and to investigate the changes occurring as a result of processing in the relevant constituents of tomato products.

1. Materials and methods

1.1. Materials

Ripe fruits of three cultivars (Rabb, Roma VF, Arab) were obtained from the same fields in the area of Wadi Ashati. The morphological characters of the fruits were:

— even round-shaped, large in size for the American cultivar Rabb,
— uneven, round-shaped, large in size for the local cultivar Arab,
— pear-shaped, medium in size for the Italian Roma VF.

The fruits were harvested from the plants under the same management, and have had the same treatments regarding fertilization, irrigation and other agricultural operations. The batches from different blocks were mixed thoroughly and divided into three replicate samples for each cultivar and stored at −20 °C when not in use.

All chemicals used were analytical grade and from BDH (London).

1.2. Technological processes

1.2.1. Tomato juice. Because of being a high yielding cultivar and of medium size, Roma VF variety was selected to be used in the technological part of this study. The above-mentioned characters are the only ones a producer prefers for processing tomatoes (STEVENS, 1979).

Three batches of the fruits were cold break-extracted, to remove skin and seeds, by means of mechanical expeller. The purees resulted were further processed by heating at 100 °C for 30 min in clean bottles closed tightly without vacuum.

1.2.2. Tomato paste. Three batches of puree were concentrated in open pans using steam jacketed kettle (Considerable amounts of tomatoes are processed locally by such method even in the pilot plants.) The concentration was continued till a product of 25% Brix value was obtained. The final product was packed mechanically into glass jars. After steam exhausting, the jars were sealed mechanically and stored at room temperature till the analyses.

1.3. Methods of analysis

1.3.1. General determinations. The pH, acidity, TS, TSS and dry matter of the fruits were determined according to the methods as described in A.O.A.C. (1980).

1.3.1.1. Determination of Vitamin C — The quantitative determination of ascorbic acid was based on its reaction with 2,6-dichlorophenol-indophenol reagents as described in details by PLUMMER (1978). The final concentration of ascorbic acid was calculated using the following equation:

$$\text{mg vitamin C/100 cm}^3 = \frac{\text{T}-\text{B1}}{\text{St}-\text{B1}} \times 2 \times \text{dilution}$$

where T, St and B1 represent the volumes of the reagent exhausted using sample, standard solution and blank, respectively.

1.3.1.2. Reducing sugars — A photometric method based on the reaction of reducing sugars with 3,5-dinitrosalicylic acid to produce permanent red colour was applied (PLUMMER, 1978). This method required a calibration curve using either glucose or fructose as standard reducing sugar.

1.3.1.3. Carotenoid pigments — Carotenoid-type pigments (mainly lycopene, β-carotene and yellow xanthophylls) were extracted according to a previously reported method (DAOOD et al., 1987). Total carotenoid pigments were estimated quantitatively by a spectrophotometric method applying the equation reported by LICHTENTHALER (1987) using extinction coefficient ($A^{1\%}_{1\,cm}$) of 2140 for conversion of absorbance readings to concentrations as follows:

$$\frac{\text{mg carotenoid}}{\text{cm}^3} = \frac{\text{absorbance at 470 nm}}{214}$$

1.3.1.4. Pectin content — Pectin content was estimated as Ca-pectate by a method depending on extraction and hydrolysis by water followed by precipitation as calcium salt (PEARSON, 1976).

1.3.1.5. Determination of iron — Determination of iron was carried out by an atomic absorption method using a model 2380 Perkin Elmer Instrument with Ion Hollow Cathod source at 248.3 nm and a mixture of 22 : 45 acetylene–-air. The calibration curve was prepared using standard solutions of different concentrations.

2. Results and discussion

2.1. Quality components of different cultivars

The suitability of tomatoes for processing is dependent to a high extent on the level of acidity, pH, total solids (TS%), total soluble solids (TSS%), total insoluble solids (TIS%), colour, etc. From the point of view of food safety, pH value of tomato products should not exceed 4.8 to avoid any possibility for some dangerous pathogenic thermophilic bacteria (e.g. *Cl. botulinum*) to grow (STEVENS, 1972). The pH values obtained for the three cultivars tested ranged

Table 1

Quality components of different cultivars of tomato for processing

Quality components	Tomato cultivars								
	Roma VF			Arab			Rabb		
	$\bar{x}$	±s	CV%	$\bar{x}$	±s	CV%	$\bar{x}$	±s	CV%
Total solids (%)	7.0	0.10	1.4	5.7	0.20	3.5	6.6	0.40	6.0
Total soluble solids (%)	4.6	0.24	5.2	4.9	0.14	2.8	6.1	0.17	2.9
Reducing sugars (% of dry matter)	65.5	2.60	3.8	80.1	5.40	6.7	50.0	1.45	2.9
pH	4.6	0.02	0.5	4.5	0.06	1.3	4.3	0.07	1.5
Acidity (mg per g dry matter)	51.7	2.15	3.4	54.6	1.70	3.1	62.8	2.15	3.4
Vitamin C (mg per g dry matter)	4.4	0.10	2.2	6.1	0.30	5.3	3.7	0.21	5.7
Pectin (% of dry matter)	2.2	0.3	13.4	3.4	0.15	4.4	7.5	1.20	16.3

$\bar{x}$: mean value of 3–4 replicates
±s : standard deviation
CV% : variation coefficient (%)

between 4.3 and 4.6 (Table 1). Such values however, are still unsuitable for safe canning unless further processing results in a considerable decrease to a value close to 4.0. In the case of pH higher than 4.0 either acidification or more drastic heat treatments would be required to avoid food poisoning. Accordingly, the cultivar Rabb showed the best pH value and organic acid content when compared with the others. Organic acid content of the fruit ranged between 0.36% and 0.41% on the basis of fresh weight. These values are in agreement with the range of 0.29% to 0.43% reported by EL-WAKEIL and KHALAF ALLAH (1987) and the range of 0.26% to 0.68% reported by BAJAJ and MAHAJAN (1982) for processing tomatoes. The observed range of acidity level appeared to be suitable for tomato processing.

As for TS% and TSS%, there was a considerable variation between different cultivars. The lowest TSS% value was recorded for Roma VF cultivar even though the TS content of the fruits was the highest. This means that the skin and seeds comprise a relatively high percentage of such fruits under the conditions of production. This unexpected result is probably due to the weak adaptation of this variety to the environmental conditions of desert locations. Favourable results were obtained from Rabb and Arab cultivars which gave fruits with TS of 6.60% and 5.70% and TSS of 6.73% and 4.99%, respectively. The latter values of TSS are generally in agreement with those reported by NASSAR and co-workers (1984) for processing tomatoes (not less than 5%) but relatively higher than found in some cultivars by EL WAKEIL and KHALAF ALLAH (1987). Since the plants were grown on the same field under the same cultural conditions and practices, variations in TSS were due to varietal dif-

ferences. This finding conformed to those of LUH anf DAOOD (1971) and EL WAKEIL and KHALAF ALLAH (1987).

Reducing sugars are of special importance in tomato processing. Most of the sugar content of ripe tomato is glucose and fructose; trace amounts of sucrose can be found in some cultivars only at early stages of ripening (BIACS et al., 1987). Reducing sugars comprise about 47% of the dry matter (GOULD, 1974). The taste of tomato fruit and products is quite dependent on the sugar content and on the sugar–acid ratio (STEVENS et al., 1977). They contribute actually to the consistency of tomato products, have a stabilizing effect on the pectin network and take part in the browning reaction enhanced by heat treatment (MEYER, 1978).

It was found that the sugar content of the cultivars examined was higher than the values mentioned in the literature for processing tomatoes probably due to the effect of sunshine period and light intensity during the growing season in desert area. Such conclusion has been achieved by GRIERSON and KADER (1986) who found strong positive correlation between light intensity and reducing sugar content of tomato fruits. Sugars came close to 80, 65.5 and 50% of dry matter in the fruits of Arab, Roma VF and Rabb varieties, respectively. These results indicate that the local Arab cultivar is suitable for fresh consumption rather than to be used for processing.

Data given in Table 1 also show the ascorbic acid concentration in fruits of the three cultivars. Ascorbic acid reached a maximum of 6.6% of dry matter in the fruits of the local variety Arab followed by Roma VF and Rabb. This vitamin ranged from 26 to 34 mg per 100 g fresh weight. This range conformed to that mentioned by RADWAN and co-workers (1979). The relatively high ascorbic acid concentration in the fruits of the local cultivar opens up the possibility to use it for preparing fresh tomato juice rich in vitamin C.

Viscosity and consistency of tomato juice are highly related to both total and soluble solid content. Pectin is one of the most important components of the total solid fraction that partakes in the consistency and relates to gross viscosity of tomato products (MARSH et al., 1980). The different cultivars showed substantial differences in their content. The highest content was found in the fruits of Rabb variety followed by Arab and Roma VF. The value estimated in Rabb cultivar (7.5% of the dry matter) was very close to the 8% reported for some processing tomatoes (STEVENS & PAULSON, 1976). The very low concentration of pectin in the fruits of Roma VF cultivar is another convincing evidence for the negative response of this cultivar to the climatic conditions by which the biochemical pathways of the biosynthesis of some important quality components was aborted.

Figure 1 shows varietal differences between the cultivars investigated for their iron and total carotenoid content. This part of our investigation was based on the concept that irrigation water of Wadi Alshati is highly contaminated

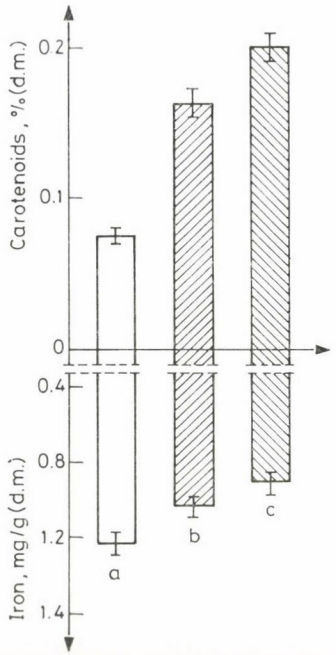


Fig. 1. Carotenoid and iron content of tomato fruit from different varieties. □: Roma VF, ▨: Arab, ▧: Rabb, ⊥: standard deviation

with iron salts (soluble and insoluble forms) which, in turn, can affect by oxidative degradation carotenoid pigment content of the fruits. The importance of such carotenoid-type pigments is coming from the responsibility of lycopene for the red colour of tomato and from vitality of β-carotene as a precursor of vitamin A. It is evident from the results that the high concentration of iron ions in the fruits of Roma VF variety is due to the effective uptake of this element by the plant whereas, the iron content of other fruits from other cultivars was in the normal range estimated in tomatoes. As for total carotenoids, there was an inverse correlation with the iron content of tomato fruits. The higher was the iron content, the lower was the carotenoid content. Such correlation may reveal the contributon of excess iron in the activation or induction of biochemical oxidation of carotenoids throughout some catalyzed reactions. The possible pathway of such degradation is the lipoxygenase-catalyzed linoleic acid oxidation which has been confirmed to be responsible for colour changes during ripening and post-harvest of tomato fruits (Daood & Biacs, 1988).

2.2. Effects of processing on quality components

2.2.1. General quality components. Table 2 shows the technologically most important components of tomato products (fresh tomato, unprocessed juice,

Table 2

Quality components of different tomato products

Quality components	Tomato products							
	fresh fruits		unheated juice		heated juice		tomato paste	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Total solids (%)	6.52	0.50	3.66	0.57	3.83	0.28	26.30	1.10
Total soluble solids (%)	3.47	0.15	3.45	0.20	3.69	0.12	25.90	1.05
Reducing sugars (% of dry matter)	45.00	1.60	48.20	1.54	35.00	4.50	73.80	2.53
pH	4.35	0.12	4.39	0.05	4.07	0.08	4.05	0.05
Acidity (mg per g dry matter)	63.00	1.60	108.50	1.82	122.30	5.20	127.10	6.40

$\bar{x}$: mean value of 4–6 replicates
$\pm s$: standard deviation

processed juice and paste). These fruits, however, were obtained from the local market before which they were stored for at least 24 h without refrigeration. This accounts for the differences in the values of the quality parameters listed in Table 2 as compared with those of the fresh fruits of the same variety whose parameters are shown in Table 1. As a result of removing skin and seeds of tomato, there was a remarkable decrease in the total solids content of juices indicating that such by-products comprise considerable percentage of the total solids in this cultivar. On the other hand, no significant change was observed in TSS content of heat treated or heat untreated tomato products except for tomato paste in which soluble solids were concentrated through water evaporation. The slight increase in Brix value of heat treated tomato juice could be attributed to solubilization of some insoluble compounds by the action of heat and acids. Such an observation has earlier been reported by LUH and DAOOD (1971) and EL WAKEIL and KHALAF ALLAH (1987).

With respect to the effect of processing on titratable acidity and pH, the obtained results revealed that titratable acidity tends to increase and correspondingly pH decreases by the effect of heat treatment. The probable explanation of such changes is that some sugars (mainly glucose) are oxidized to uronic acid, in the presence of molecular oxygen, by heat-catalyzed reactions (BERK, 1976). Such a proposition is supported by the remarkable decrease in reducing sugar content accompanied by an increase in titratable acidity of the heat-treated juice. Similar results have been obtained by many workers, but explanation was obscure (HAMDY & GOULD, 1962; EL WAKEIL & KHALAF ALLAH, 1987). Further heating to process tomato paste caused only a slight increase in titratable acidity emphasizing the essentiality of soluble or molecular oxygen in the above mentioned reactions (oxygen is removed through further condensation of tomato puree in open pans).

Regarding reducing sugars, severe degradation was found in the sample of juice processed in closed bottle (without vacuum). The presence of oxigen was thought to be responsible for the drastic impairment of sugars in this product. The role of oxygen could be seen in aldose to alduronic conversion and/or in heat initiated non-enzymatic browning reactions (Maillard types) (MEYER, 1978). In the case of tomato paste, high accumulation of reducing sugars was recorded. This could be attributed to the hydrolysis of polysaccharides by the action of heat and acids.

2.2.2. Changes in ascorbic acid. Beside vitamin C activity of ascorbic acid it plays an important role as natural antioxidant in fruit and vegetable products (DAOOD et al., 1989; BIACS et al., 1988). However, during processing of fruits and vegetables, ascorbic acid contributes actively to browning through a series of lactone and furfural formation reactions (MEYER, 1978; BERK, 1976) leading to an impairment in the quality of the product.

As regards the susceptibility of ascorbic acid during tomato processing (Fig. 2), cold break extraction using metal expeller machine this was found to be the first step of tomato processing by which a great destruction of ascorbic acid happens. Tomato juice lost about 70% of the original amount of ascorbic acid when extracted in this way. Trapping of molecular oxygen as a result of whipping and foam formation is the reasonable cause of high destruction since ascorbic acid is very sensitive to oxygen and oxidizing elements. Therefore, it is necessary to use machines made of stainless steel and to minimize foaming of tomato puree in order to reduce oxidative degradation of vitamin C. Depending

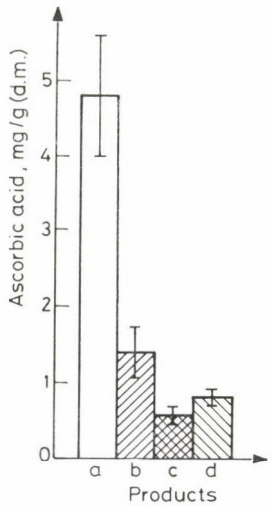


Fig 2. Ascorbic acid content of different products of tomato. □: Fresh tomato, ▨: unheated juice, ▬: heated juice, ▧: tomato paste, ⊥: standard deviation

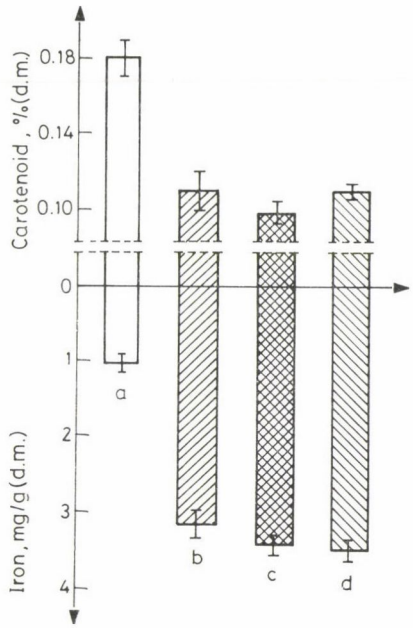


Fig. 3. Carotenoid and iron content of different products of tomato. For symbols see Fig. 2

on the presence or absence of oxygen heat processes vary in their influence on ascorbic acid. Heating tomato juice in closed bottles, where oxygen could not be removed, resulted in a product with the lowest ascorbic acid content, whereas, further processing to produce tomato paste in an open pan had no such drastic effect on vitamin C; probably due to the removal of oxygen and to the preserving influence of some ingredients such as sugars, salts and insolubles concentrated by evaporation of water. The relatively high ascorbic acid contents of tomato pastes as compared with other processed products, have been reported by GOULD (1974).

2.2.3. Effects of processing on carotenoids and iron. The most stable carotenoid pigments of tomato fruits are those of low polarity (lycopene and carotenes). Nevertheless, oxidative destruction of such compounds is induced by metal, light, heat and oxygen-catalyzed reactions. The severity of destruction is quite dependent on strength of the catalysts and heat process.

Like ascorbic acid, total carotenoids decreased dramatically after extraction by metal extractor. This impairment was accompanied by remarkable increase in iron content of the extracted puree (Fig. 3). The source of iron contamination of tomato juice is not only the metal expeller but also washing water which is known to contain high amounts of iron. Since further destruction of carotenoid in heat-processed products depended on the presence of oxygen, it could be concluded that oxygen is essentially needed beside the other catalysts

for the induction of such destruction. As for the iron content of heat-processed products, there was a slight increase probably due to the release of organic iron as a results of hydrolysis processes.

3. Conclusions

It is of great importance to emphasize that studies in genetics will be indispensable and promise better hybrid varieties of tomatoes which suit the climatic conditions of the desert and help to satisfy the farmers beside possessing characteristics agreeable to the demands of technology.

Regarding the effect of processing on the quality components of tomato, mechanical extraction and pasteurization of extracted purees in the presence of oxygen brings about an impairment in the quality of tomato products. When compared to the fresh fruit, unheated and heated juices showed significant differences in the quality components. LSD calculations for TS%, TSS%, reducing sugar content, pH and acidity give $LSD_{5\%}$ values of 0.46 not significant, 2.8, not significant and 8.4, respectively. It could also be concluded from this work that the severe destruction of vitamin C and carotenoid pigments in tomato products is related more to the trapping of oxygen and the presence of iron in the juice than to heat treatment.

Literature

A. O. A. C. (1980): Official methods of analysis. Association of Official Analytical Chemists, Washington 25 D. C. USA.

AUNG, L. H. (1979): Temperature regulation of growth and development of tomato during antogeny. — In: *Proceeding of 1st International Symposium on Tropical Tomato.* Asian Vegetable Research and Development Centre. Shanhua Taiwan, China. pp. 79–93.

BAJAJ, K. L. & MAHAJAN, R. (1982): Chemical constituents of fruits of some rootknot nematode resistant varieties of tomato. *J. Res. Punjab Agric. Univ., 19,* 339–345.

BERK, Z. (1976): *Braverman's introduction to the biochemistry of foods.* Elsevier, Amsterdam.

BIACS, P., DAOOD, H. G., CZINKOTAI, B., HAJDU, F. & KISS-KUTZ, N. (1987): Effect of Titavit treatment on the dynamics of tomato fruit ripeness, *Acta Hortic., 220,* 433–438.

BIACS, P., DAOOD, H. G., PAIS, I., FEHÉR, M. & HAJDU, F. (1988): Chromatographic investigation of tomato fruit metabolites during ripening. — In: HUBA, K. & ETTRE, L. (Eds) *Chromatograph—87.* Akadémiai Kiadó. Budapest, pp. 39–59.

DAOOD, H. G., BIACS, P., HOSCHKE, A., VINKLER, M. & HAJDU, F. (1987): Separation and identification of tomato fruit pigments by TLC and HPLC. *Acta Alimentaria, 16,* 339–350.

DAOOD, H. G. & BIACS, P. (1988): Some properties of tomato fruit lipoxygenase. *Acta Alimentaria, 17,* 53–65.

DAOOD, H. G., BIACS, P., KISS-KUTZ, N., HAJDU, F. & CZINKOTAI, B. (1989): Lipids and antioxidant content of red pepper. — In: BIACS, P., GRUIZ, K. & KREMMER, T. (Eds) *Biological role of plant lipids.* Akadémiai Kiadó, Budapest and Plenum, New York, pp. 491–494.

EL WAKEIL, F. A. & KHALAF ALLAH, A. M. (1987): Evaluation of some new tomato cultivars. — Part II. Suitability for juice production. *Acta Hortic., 220,* 447–452.
FARKAS, J. (1987): Breeding for tomato fruit quality. *Acta Hortic., 220,* 69–75.
GOULD, W. A. (1974): *Tomato production, processing and quality evaluation.* The AVI Pub. Co. Inc., Westport, Conn., USA.
GRIERSON, D. & KADER, A. A. (1986): Fruit ripening and quality. — In: ATHERTON, J. G. & RUDISH, J. (Eds) *The tomato crop.* Chapman and Hall, London. pp. 241–280.
HAMDY, M. M. & GOULD, W. A. (1962): Varietal differences in tomatoes: A study of alpha-keto acids, alpha amino compounds and citric acid in eight tomato varieties before and after processing. *J. agric. Fd Chem., 10,* 499–504.
LICHTENTHALER, H. K. (1987): Chlorophylls and carotenoids. Pigments of photosynthetic biomembranes. *Meth. Enzym., 18,* 350–382.
LUH, B. S. & DAOOD, H. N. (1971): Effect of break temperature and holding time on pectin and pectic enzyme in tomato pulp. *J. Fd Sci., 36,* 1039–1042.
MARSH, G. L., BUHLERT, J. E. & LEONARD, S. J. (1980): Effect of composition upon Bostwich consistency of tomato concentrate. *J. Fd Sci., 45,* 703–708.
MEYER, L. H. (1978): *Food chemistry.* The AVI Pub. Co. Inc., Westport, Conn., USA.
NASSAR, S. H., SIMS, W. L. & HASSAN, A. A. (1984): Nationwide programme of tomato cultivar evaluation in Egypt: 1980–1982 trials. *Egypt. J. Hortic., 11,* 163–190.
PEARSON, D. (1976): The chemical analysis of foods. (7th ed.) Churchill Livingstone Pub. Edinburgh, p. 159.
PLUMMER, D. T. (1978): *An introduction to practical biochemistry.* (2nd ed.) McGraw Hill Book Co. UK.
RADWAN, A. A., HASSAN, A. A. & MALASH N. M. (1979): Correlation studies on tomato fruit firmness, total soluble solids and vitamin C content. *Fac. Agric. Ain Shams Univ. Res. Bull., No. 1063,* 17.
STEVENS, M. A. (1972): Relationship between components contributing to quality variation among tomato lines. *J. Amer. Soc. Hortic. Sci., 97,* 70–73.
STEVENS, M. A. (1979): Breeding tomato for processing. — In: *Proceedings of the 1st International Symposium on Tropical Tomato.* Asian Vegetable Research and Development Centre. Shanhua, Taiwan. pp. 201–213.
STEVENS, M. A. (1986): Inheritance of tomato fruit quality components. *Plant Breeding Rev., 4,* 274–311.
STEVENS, M. A., KADER, A. A., ALBRIGHT-HOLTON, M. & ALGAZI, M. (1977): Genotypic variation for flavour and composition in fresh market tomatoes. *J. Amer. Soc. Hortic. Sci., 102,* 680–689.
STEVENS, M. A. & PAULSON, K. N. (1976): Contribution of components of tomato fruit alcohol insoluble solids to genotypic variation in viscosity. *J. Amer. Soc. Hortic. Sci., 101,* 91–96.

Acta Alimentaria, Vol. 19 (4), pp. 359–376 (1990)

QUALITY CHANGES OF ORANGE SOFT DRINKS DURING STORAGE

I. VARSÁNYI and L. SOMOGYI

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 5 February 1990; accepted: 13 April 1990)

Changes occurring in the quality of 3 orange drinks — one juice with natural aroma and two samples aromatized with synthetic aroma — was studied during storage. The samples were kept in bottles of 200 cm^3 closed with crown cap, as used on the market. The bottles were stored at two different temperatures (5 °C, 20 °C) in dark and in daylight, respectively. Quality changes were followed up by sensory scoring tests. The results were evaluated by mathematical-statistical methods and grafically presented. The critical sensory property was selected by covariance analysis.

The characteristic critical property most rapidly changing was found to be the smell or the taste. The effect of light, particularly with drinks of natural aroma, was found to be determinative.

The quality change of the natural juice was generally more rapid than that of aromatized drinks. Soft drinks prepared with synthetic aroma are more resistant to conditions of storage (temperature, light).

The comparison of functions describing change (deterioration) has shown that natural juices of higher nutrition-biological value keep their quality in bottle protecting them from light.

Keywords: soft drinks, shelf life, quality changes of orange beverages, soft drinks packaging

Food quality normally deteriorates during storage, exceptions are rare. Changes of quality can be traced back to microbiological, physical and chemical effects. The deterioration cannot generally be connected to the effect of a single factor, it derives mostly from the joint effects of chemical, physical or even microbiological factors as shown in the literature (FARKAS et al., 1975; VARSÁNYI & SOMOGYI, 1982; LABUZA, 1983; VARSÁNYI, 1985).

Since changes occurring in the quality of foods are most sensitively indicated by their sensory properties, it is desirable to follow them up beyond the instrumental analyses, by sensory tests, too. The necessity of estiblishing sensory changes is supported also by the fact that the consumer judges food by his sensory experience and, thus, these properties are considered competent by him.

Therefore, our experiments were aimed at the development of a deterioration model by which the change in marketed drinks of different composition (aroma) and stored under different conditions can be characterized or described.

Quality changes are mostly affected by the initial quality of the food, its composition, conditions of storage (temperature, light, relative humidity, etc.) and the packaging (KAREL et al., 1971; QUAST & KAREL, 1973; VARSÁNYI, 1987).

Out of the storage conditions the role of temperature, affecting both the chemical reactions and microbiological processes, is important. The rate of chemical reactions increases exponentially with increasing temperature and so does the multiplication of microorganisms, too (SIMON et al., 1982; LABUZA & RIBOH, 1982).

It is known also that light absorbed by the medium may substantially influence the oxidation–reduction reactions and thereby affect quality change. Particularly important is therefore the effect of light in the case of different drinks (BASETTE, 1976; MAN, 1978; VARSÁNYI, 1988).

Packaging is important because it may greatly reduce the detrimental effect of environment. In the case of drinks, juices, for instance, the catalytic effect of light upon change of quality during storage can be reduced or completely eliminated. The possibility of perfect protection (no permeability) inhibits oxidation processes.

Of all the factors influencing quality change the most important is the length of storage period. Foods are considered intricate biological–chemical––physical systems in which as a function of storage time irreversible changes take place. In view of the changes of the parameters characteristic of their quality as a function of storage time the interpretation and description of stochastic correlations between quality and time, is indicated (GACULA & KUBALA, 1975).

In the present study the change as a function of time in the sensory properties of three different orange drinks, stored under different conditions, was investigated.

It was our aim to establish how the sensory properties of orange soft drinks, found on the market change in identical packaging but under different storage conditions. Results were evaluated by mathematical-statistical methods in order to obtain objective conclusions.

1. Materials and methods

1.1. Materials

The samples were soft drinks obtained on the market and aromatized with three different orange aroma substances. Sample *A* was prepared with natural orange aroma, sample *B* represented a diluted orange juice concentrate and sample *C* was aromatized with synthetic aroma. The three samples were filled uniformly in 200 cm^3 bottles and closed with the crown cap.

1.2. Methods

The storage conditions applied were similar to those most frequently found in commercial practice, thereby providing useful information for the trade, too. The bottles containing the samples were stored at +5 °C in the dark and at 20 °C temperature in the dark and in light, respectively.

The samples used in the storage experiment were taken randomly from industrial products available on the market. The same number of samples was used for each storage condition. Control tests, depending on the conditions of storage, were carried out at least five times to the time of deterioration.

The change of quality was followed up by sensory tests. The sensory tests were carried out according to HUNGARIAN STANDARD (1974) in two repetitions. The panel of judges consisted of 5 to 9 members. The 20 point system using weighting factors was applied, scoring the following characters: appearence (weighting factor 0.6); colour (w. f. 0.6); smell (w. f. 0.8); taste (w. f. 2.0).

The weighted total score was obtained by averaging the values of the two parallel tests. The product is considered of standard quality if the average score of each group of properties amounted to at least 2.8 points or the weighted total score amounted to 11.2.

Results were evaluated by mathematical-statistical methods. By comparing the results of tests carried out to follow up quality changes the critical parameter or the parameter which change most rapidly as a function of time, was established. The rate of change of the critical parameter was studied and evaluated in relation to changes in the storage conditions.

To follow quality change the suitable function was determined by regression analysis — if necessary with linearized data — since the data show normal distribution and the variances were homogeneous. The accuracy of curve fitting was characterized by the correlation coefficient related to the linearized data. The mean values and the deviation of the two parameters as probability variables of the curve of deterioration were also calculated. If the deviation of the two parameters exceeds 30% of the average another form of function must be used to describe the quality change. To compare the curves paired covariance analysis was used (KÖRMENDY, 1983).

Calculations were based on the following relationship. Linearized formula of the curve of deterioration:

$$y = a + bx$$

where

y = value of the quality characteristic (expected value),
a = characteristic value of quality at the beginning of storage,
b = constant of the rate of quality change,
x = storage period.

The values of deviations belonging to a and b parameters were calculated by the following equations:

$$S_a = \frac{\sum e_i^2 - \sum x_i^2}{n(n-2)\sum (x_i - \bar{x})^2}$$

$$S_b = \frac{\sum e_i^2}{(n-2)\sum (x_i - \bar{x})^2}$$

where

$e = y - y^*$: deviation of the dependent variable from the assessed value,
y^* = assessed value of the dependent variable,
x = mean value of the independent variable,
n = number of measurements.

Accuracy of curve fitting was characterized by the value of correlation coefficient (r) according to Bravais.

$$|r| = \frac{\sum (y^* - \bar{y})^2}{\sum (y - \bar{y})^2}$$

where

$\bar{y}$ = average of the values characterizing quality as measured,
y = value characterizing quality as measured at x point of time.

The model of covariance analysis applicable to the comparison of deterioration curves can be calculated by the F test related to the zero hypothesis (H_0) as follows.

$$H_0 = \frac{V_1}{V_0} < F_p$$

where

V_1 = variance of linear regression,
V_0 = variance of the common linear regression replacing the two curves
F_p = critical F value as calculated at p(%) probability level.

2. Results

The results of the sensory analysis of the soft drinks prepared with three aromatic substances of different origin are summarized as follows. Figures 1, 2, 3 show the change with time of the sensory properties of the soft drink prepared with natural aroma.

Figure 1 shows the quality change as a function of time in the product stored at 20 °C exposed to light. As seen in the Figure out of the sensory properties smell, taste and colour changed in a nearly identical way during the storage time.

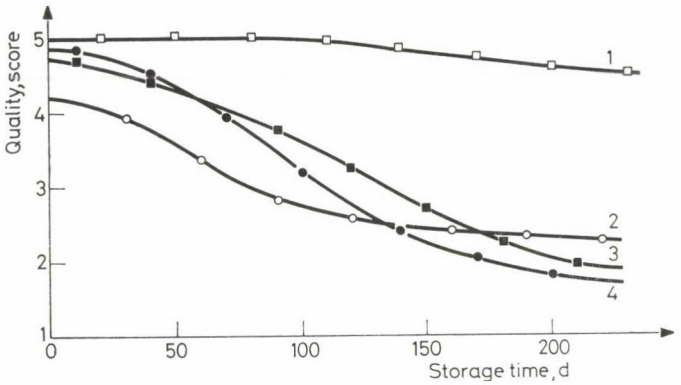


Fig. 1. Changes in sensory properties of orange juice made by natural aroma stored in light at 20 °C. 1: Appearance, $y = 5.0\ e^{-10^{-6}x^2}$; 2: colour, $y = 4.2\ e^{-0.07x^2}$; 3: smell, $y = 4.6\ e^{-0.05x^2}$; 4: taste, $y = 4.7\ e^{-0.06x^2}$

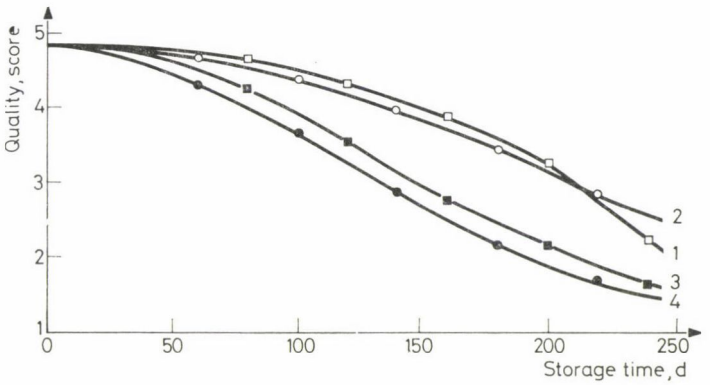


Fig. 2. Changes in sensory properties of orange juice made by natural aroma stored in dark 20 °C. 1: Appearance, $y = 4.8\ e^{-0.04x^2}$; 2: colour, $y = 4.8\ e^{-0.04x^2}$; 3: smell, $y = 4.8\ e^{-0.0242x^2}$; 4: taste, $y = 4.8\ e^{-0.02x^2}$

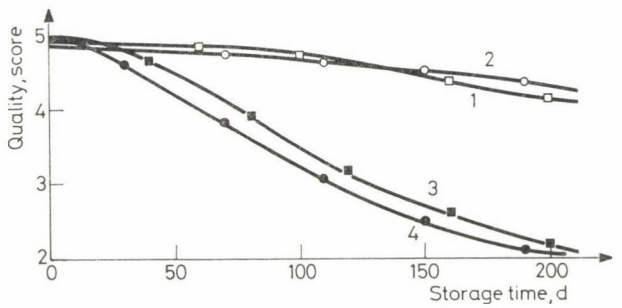


Fig. 3. Changes in sensory properties of orange juice made by natural aroma stored in dark at 5 °C. 1: Appearance, $y = 5.0\ e^{-10^{-8}x^2}$; 2: colour, $y = 5.0\ e^{-10^{-8}x^2}$; 3: smell, $y = 5.0\ e^{-0.06x^2}$; 4: taste, $y = 5.0\ e^{-0.05x^2}$

In Fig. 2 the curves of the product stored at 20 °C in the dark are shown. As shown by the curves the colour and appearence changes at a slower

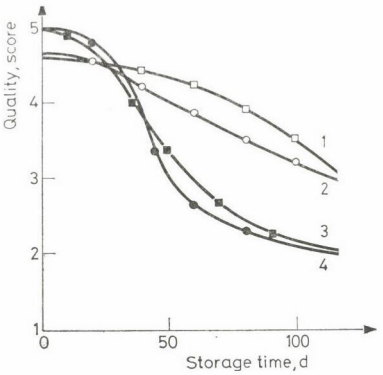


Fig. 4. Changes in sensory properties of natural orange juice stored in light at 20 °C 1: Appearance, $y = 4.6\ e^{-0.09x^2}$; 2: colour, $y = 4.7\ e^{-0.08x^2}$; 3: smell, $y = 5.0\ e^{-0.2x}$ 4: taste, $y = 5.0\ e^{-0.18x^2}$

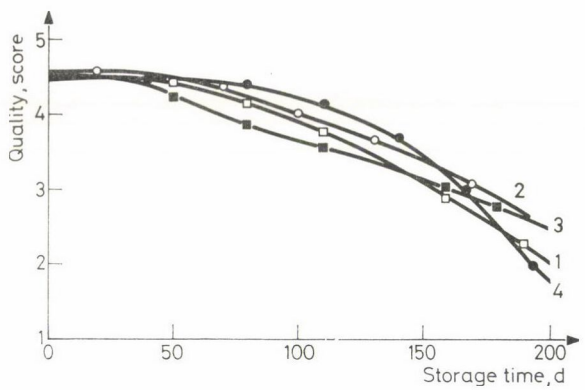


Fig. 5. Changes in sensory properties of natural orange juice stored in dark at 20 °C 1: Appearance, $y = 4.6\ e^{-0.002x^2}$; 2: colour, $y = 4.5\ e^{-0.001x^2}$; 3: smell, $y = 4.3\ e^{-0.0015x^2}$; 4: taste, $y = 4.5\ e^{-0.0018x^2}$

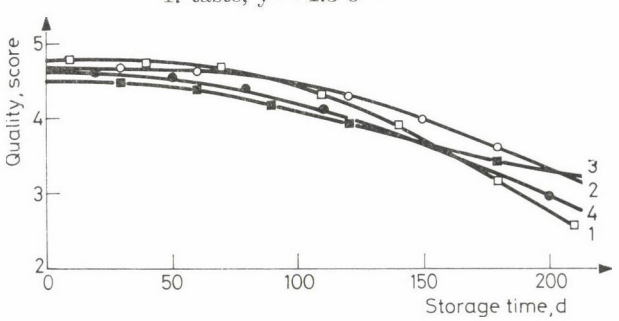


Fig. 6. Changes in sensory properties of natural orange juice stored in dark at 5 °C. 1: Appearance, $y = 4.6\ e^{-0.002x^2}$; 2: colour, $y = 4.5\ e^{-0.001x^2}$; 3: smell, $y = 4.3\ e^{-0.0015x^2}$; 4: taste, $y = 4.5\ e^{-0.0018x^2}$

rate than smell and taste. These latter two properties behave similarly during storage.

In Fig. 3 the quality change curves of the product stored at 5 °C in the dark are shown. As it is well seen, the change of the quality characteristics forms two distinct groups. One group is formed by the colour and appearence while the other by smell and taste. The rate of change in the two groups is different.

Figures 4, 5 and 6 show the deterioration curves of the product prepared by diluting a natural fruit juice concentrate and storing it under different conditions.

In Fig. 4 the quality change curves of the product stored at 20 °C exposed to light, are shown. As seen, the rate of change in colour and appearence is similar, while that of smell and taste, though very similar to one another, is quite different from the other group.

In Fig. 5 the deterioration curves of the diluted fruit concentrate stored at 20 °C in the dark, are shown. All the sensory properties seem to behave similarly under these storage conditions.

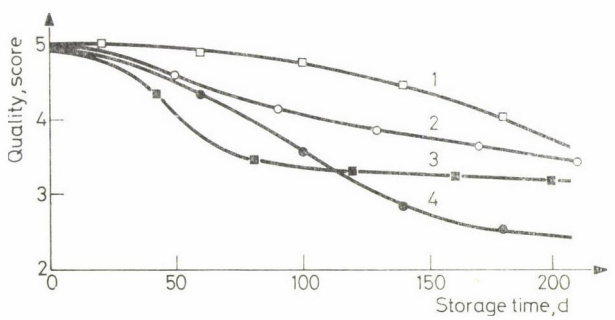


Fig. 7. Changes in sensory properties of orange juice made by artificial aroma, stored in light at 20 °C. 1: Appearance, $y = 5.0\ e^{-0.0005x^2}$; 2: colour, $y = 5.0\ e^{-0.001x^2}$; 3: smell, $y = 4.9\ e^{-0.008x^2}$; 4: taste, $y = 4.9\ e^{-0.0055x^2}$

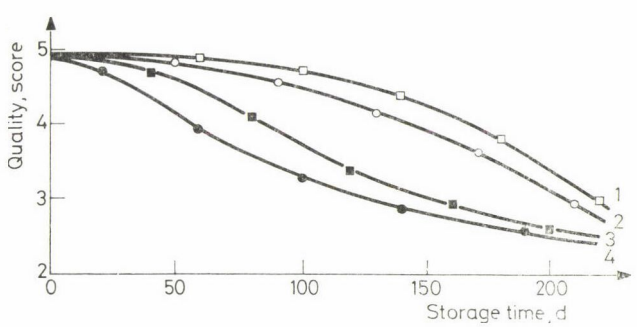


Fig. 8. Changes in sensory properties of orange juice made by artificial aroma stored in dark at 20 °C. 1: Appearance, $y = 4.8\ e^{-0.03x^2}$; 2: colour, $y = 4.8\ e^{-0.0035x^2}$; 3: smell, $y = 4.8\ e^{-0.004x^2}$; 4: taste, $y = 4.8\ e^{-0.005x^2}$

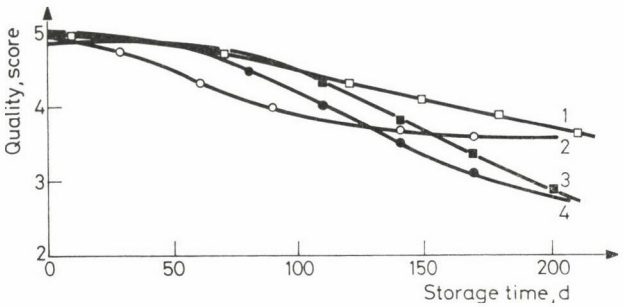


Fig. 9. Changes in sensory properties of orange juice made by artificial aroma stored in dark at 5 °C. 1: Appearance, $y = 5.0\ e^{-10^{-7}x^3}$; 2: colour, $y = 5.0\ e^{-10^{-6}x^3}$; 3: smell, $y = 4.8\ e^{-0.0043x^2}$; 4: taste, $y = 5.0\ e^{-0.0038x^2}$

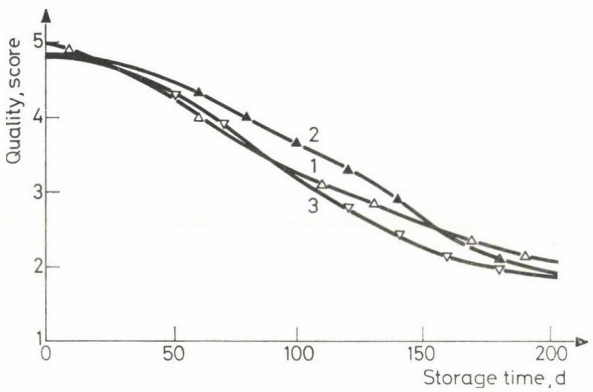


Fig. 10. Changes in taste of orange juice made by natural aroma stored under different conditions. 1: 5 °C, dark: $y = 5.0\ e^{-0.05x^2}$; 2: 20 °C, dark: $y = 4.8\ e^{-0.02x^2}$; 3: 20 °C, light: $y = 4.7\ e^{-0.06x^2}$

In Fig. 6 the quality change curves of the diluted fruit juice, stored at 5 °C in the dark, are given. The rate of change of the four sensory properties seems to be nearly identical.

Figures 7, 8 and 9 show the deterioration curves of the soft drink aromatized with synthetic aroma substances stored under different conditions.

In Fig. 7 the quality change curves of the product stored at 20 °C and exposed to light, are shown. As shown by the curves smell and taste change in a nearly identical way as a function of time, while the other properties in quite a different way.

In Fig. 8 the change of the product stored at 20 °C and in the dark, are shown. As seen in the figure smell and taste of the product change nearly identically, while the other two sensory properties change slower.

Figure 9 contains the curves illustrating the change in the sensory properties of the drink stored at 5 °C in the dark. In this case, too, appearence

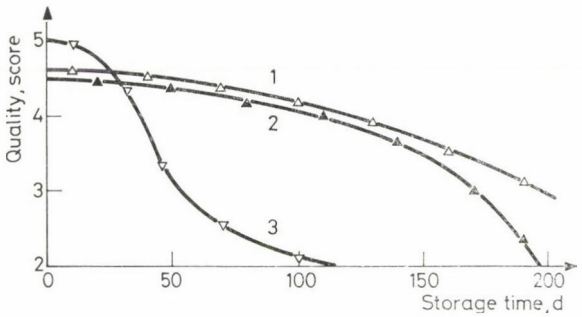


Fig. 11. Changes in taste of natural orange juice stored under different conditions. 1: 5 °C, dark: $y = 4.5\ e^{-0.0018x^2}$; 2: 20 °C, dark: $y = 4.5\ e^{-0.084x^2}$; 3: 20 °C, light: $y = 5.0\ e^{-0.18x}$

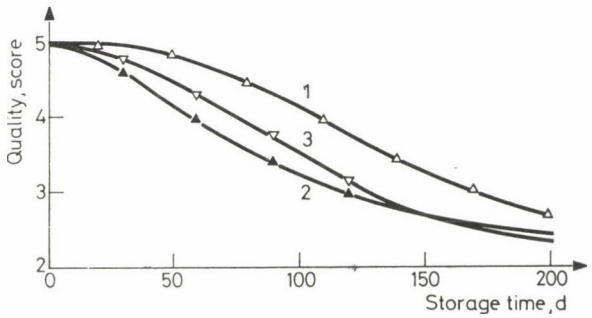


Fig. 12. Changes in taste of orange juice made by artificial aroma stored under differen, conditions. 1: 5 °C, dark: $y = 5\ e^{-0.0038x^2}$; 2: 20 °C, dark: $y = 4.8\ e^{-0.005x^2}$; 3: 20 °C, light: $y = 4.9\ e^{-0.0055x^2}$

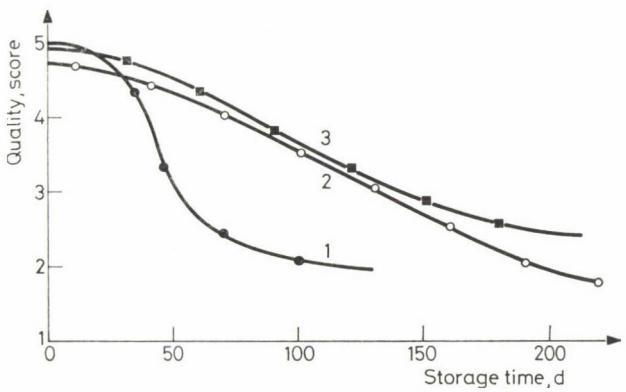


Fig. 13. Changes in taste of the orange juices stored in light at 20 °C. 1: Natural juice $y = 5.0\ e^{-0.18x^2}$; 2: juice made by natural aroma, $y = 4.7\ e^{-0.06x^2}$; 3: juice made by artifical aroma, $y = 4.9\ e^{-0.0055x^2}$

and colour change in a similar way. The change of smell and taste is also similar, but differing from the other two properties.

The samples aromatized in the same way, but kept under different storage conditions are illustrated in Figs. 10, 11 and 12. Comparison was carried out with the curves showing the change in taste since this changed in a way characteristic of spoilage under every condition of storage.

In Fig. 10 the change in taste of the product aromatized with natural aroma and stored under different conditions was compared. The curves seen in the Figure show a nearly identical change as a function of time.

In Fig. 11 the change of taste in the fruit juice is illustrated. It can be seen that the curve of the juice stored at 20 °C in light differs significantly from the other two curves.

Figure 12 shows the change of taste in the product prepared with synthetic aroma as a function of storage time. The curve illustrating the change of

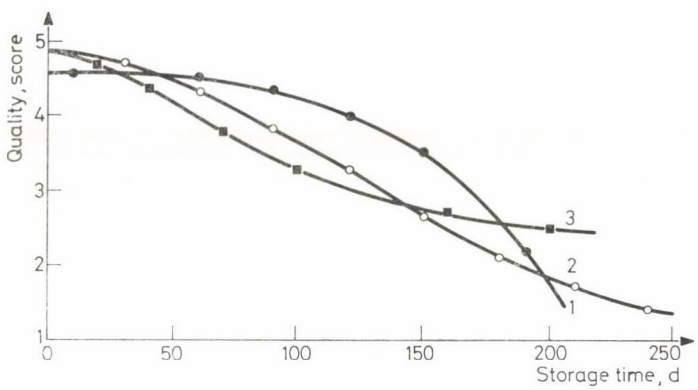


Fig. 14. Changes in taste of the orange juices stored in dark at 20 °C. 1: Natural juice, $y = 4.5\ e^{-0.084x^2}$; 2: Juice made by natural aroma, $y = 4.8\ e^{-0.02x^2}$; 3: Juice made by artifical aroma, $y = 4.8\ e^{-0.005x^2}$

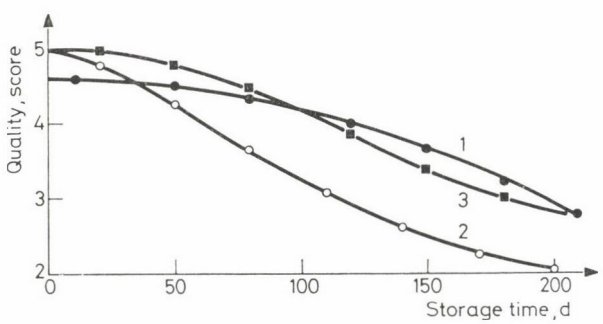


Fig. 15. Changes in taste of the orange juices stored in dark at 5 °C. 1: Natural juice, $y = 4.5\ e^{-0.0018x^2}$; 2: Juice made by natural aroma, $y = 5.0\ e^{-0.05x^2}$; 3: Juice made by artificial aroma, $y = 5.0\ e^{-0.0038x^2}$

taste in the sample kept at 5°C in the dark is substantially less steep than the other two curves, which are almost identical.

The effect of aromatization upon the character of quality change is compared in Figs. 13, 14 and 15.

In Fig. 13 the change of taste in the sample stored at 20 °C in light, is illustrated. It can be well seen that the curve of the product prepared with synthetic aroma is steeper than the curves of the other two products.

In Fig. 14 the change of taste in the products stored at 20 °C in the dark, is illustrated. The curves showing the change of the three different products are different, that is, the rate of change is different in each of the three products.

In Fig. 15 the deterioration curves of the three products stored at 5 °C in the dark, are illustrated. Depending on the aromatization the rate of change is different as shown by the three curves.

3. Conclusions

The analysis of quality change in the three different orange soft drinks has shown that the change can be described under all storage conditions (at 5 and 20 °C, in light and in the dark) by the following type of function.

$$y = a\, e^{bx^2}$$

where

y = value of the quality change characteristic to the product after the storage period,
x = the storage period,
a = value of the quality characteristic at the starting of storage,
b = characteristic of the constant rate of deterioration.

Differential form of the function:

$$\frac{dy}{dx} = 3bxy$$

where

$\frac{dy}{dx}$ factor of the rate of quality change.

The rate of quality change — in accordance with the above correlation — is determined by two factors: storage period and the rate of reactions causing the change of quality. The joint effect of the two factors lends a characteristic "sigmoid" shape to the curve describing quality change.

Table 1

Comparison of the results of experiments carried out to establish quality changes in the orange drink prepared from natural fruit juice stored under different conditions

Quality character	Storage conditions 20 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	Ø	Ø	—	
Taste	Ø	Ø	Ø	—

Quality character	Storage conditions 20 °C, in light			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	**	**	—	
Taste	**	**	Ø	—

Qua ity character	Storage conditions 5 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	Ø	Ø	—	
Taste	Ø	Ø	Ø	—

Ø = non significant ($P < 95\%$)
** = highly significant ($P = 99\%$)

The deterioration curves thus described were compared by covariance analysis in order to be able to estimate the effect of storage conditions and aromatization upon the rate of quality change.

Table 1 contains the data of the orange soft drink prepared with natural aroma and stored under different conditions and obtained by covariance analysis of their deterioration curves. As seen in the table the difference in the rate of change in appearence and colour as well as smell and taste is not significant. The rate of change in quality of appearence and colour differ from that of smell and taste at the 95% and 99% probability level, respectively. Thus, the calculations have shown that from the point of view of the change of appearence and colour form a group and smell and taste from another group.

Table 2

Comparison of the results of experiments carried out to establish quality changes in orange drink prepared with natural aroma and stored under different conditions

Quality character	Storage conditions 20 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	**	*	—	
Taste	**	**	Ø	—

Quality character	Storage conditions 20 °C, in light			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	**	—		
Smell	**	Ø	—	
Taste	**	Ø	Ø	—

Quality character	Storage conditions 5 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	***	***	—	
Taste	***	***	Ø	—

Ø = non-significant ($P \leq 95\%$)
* = significant ($P \geq 95\%$)
** = highly significant ($P \geq 99\%$)
*** = very highly significant ($P \geq 99.9\%$)

The latter two quality characteristics show a significantly higher deterioration rate than the former ones, therefore these characteristics can be considered critical.

With products stored at 20 °C in light the rate of quality change in colour, smell or taste did not differ significantly. However, the rate of change in appearence differed from that of the former ones significantly ($P \geq 0.95\%$). As regards change colour, smell and taste showing a higher rate of change can be considered the critical characters.

The statistical comparison of the deterioration curves of product stored at 5 °C in the dark showed that the rate of change in appearence and colour

on one hand and in smell and taste on the other, did not differ significantly. The difference between the two groups, however, proved to be significant at the 99% probability level. Thus, in this case, too, the change in smell and taste can be considered critical.

Summing up the results in Table 2 it can be said that the quality change of orange juice prepared with natural aroma can be characterized by smell and taste independently from the conditions of storage. However, the joint effect of light and temperature accelerates colour change, too.

In Table 2 the results of the statistical analysis of data of quality change in orange drink obtained by deluting natural orange juice concentrate and storing it under different conditions, are summarized. The results in the Table show that the rate of deterioration in the product stored in the dark at either temperature can be considered identical. In the sample stored at 20 °C in light the deterioration rate of smell and taste was found significantly different at the probability level of 95% from the deterioration rate of appearence and colour. In this case, from the aspect of quality change smell and taste are considered the critical characters.

The conclusion drawn from the results in Table 2 is that the quality change of orange soft drink prepared by deluting natural orange juice concentrate is mainly influenced by light. The deterioration rate is substantially reduced if the product is stored in the dark, independently from the applied temperature.

Table 3 contains the results of the statistical analysis of data of change of quality in orange drink prepared with synthetic aroma and stored under different conditions.

As seen from the results no significant difference was observed in the change of appearence or colour in the drink stored at 20 °C in the dark. The same observation was made in relation to the change of smell and taste. Thus, the quality characters fall into two groups: the change in smell and taste differs from that in appearence and colour at the probability level of 90%, the rate of change is that much higher, therefore, these two characters can be considered the critical ones.

In the product stored at 20 °C in light the rate of quality change of smell and taste did not differ significantly, however, the rate of change was significantly higher than that of the other two characters. In this case, the rate of change in appearence and colour differed, too, however, in relation to change of quality, smell and taste are considered critical.

In the product stored at 5 °C in the dark the rate of quality change in smell and taste differed from that in the other two sensory characters, appearence and colour. The difference between the two groups became apparent at the 90% probability level, accordingly smell and taste are the characters considered critical for deterioration.

Table 3

Comparison of the results of experiments carried out to establish quality changes in orange drink prepared with synthetic aroma during storage under different conditions

Quality character	Storage conditions 20 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	*	*	—	
Taste	*	*	Ø	—

Quality character	Storage conditions 20 °C, in light			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	*	—		
Smell	**	**	—	
Taste	**	*	Ø	—

Quality character	Storage conditions 5 °C, in the dark			
	Appearance	Colour	Smell	Taste
Appearence	—			
Colour	Ø	—		
Smell	*	*	—	
Taste	*	*	Ø	—

Ø = non-significant ($P \leq 95\%$)
* = significant ($P \geq 95\%$)
** = highly significant ($P \geq 99\%$)

Results shown in Tables 2, 3 and 4 permit the conclusion that the changes of smell and taste are sensitive indicators of the quality change, therefore, in this study of orange drinks these two sensory characters have proven critical of quality.

The next step in this investigation was to establish the effect of storage conditions upon the change of taste, considered the critical character. Results are given in Table 4.

It can be seen in the Table that the change of taste in the orange drink containing natural aroma did not show significant difference as an effect of the different storage conditions.

Table 4

Results obtained by statistical comparison of the change in taste of orange drinks stored under different conditions

Storage conditions	Aroma substances: Natural aroma			Natural fruit juice			Synthetic aroma		
	20 °C, in the dark	20 °C, in light	5 °C, in the dark	20 °C, in the dark	20 °C, in light	5 °C, in the dark	20 °C, in the dark	20 °C in light	5 °C, in the dark
+20 °C, in the dark				—			—		
+20 °C, in light	Ø	—		***	—		Ø	—	
+ 5 °C, in the dark	Ø	Ø	—	**	***	—	*	*	

Ø = non-significant ($P \leq 95\%$)
* = significant ($P \geq 95\%$)
** = highly significant ($P \geq 99\%$)
*** = very highly significant ($P \geq 99.9\%$)

Table 5

Calculated results obtained by statistical comparison of changes of taste in orange drinks prepared with different aroma substances

Quality parameters	Storage conditions: 20 °C, in the dark			20 °C, in light			5 °C, in the dark		
	natural aroma	natural fruit juice	synthetic aroma	natural aroma	natural fruit juice	synthetic aroma	natural aroma	natural fruit juice	synthetic aroma
Natural aroma	—			—			—		
Natural fruit juice	**	—		***	—		**	—	
Synthetic aroma	**	**	—	Ø	***	—	**	*	—

Ø = non-significant ($P \leq 95\%$)
* = significant ($P \geq 95\%$)
** = hihgly significant ($P \geq 99\%$)
*** = very highly significant ($P \geq 99.9\%$)

The taste of the orange drink prepared from orange juice showed the most rapid deterioration when stored at 20 °C in light and it differed from the rate of change in the other two samples, stored at 5 and 20 °C, respectively, in the dark, at the 99% probability level. The lowest rate of change was shown by the sample stored at 5 °C in the dark.

As regards the drink prepared with synthetic aroma in the sample stored at 20 °C, whether in light or in the dark, the rate of change of taste did not differ significantly. However, the change of taste in the sample stored at 20 °C differed at 90% probability level from that stored at 5 °C, the rate of change being significantly higher.

The calculations as summarized in Table 4 permit the conclusion that the orange drink prepared from natural fruit juice is more sensitive to environ-

mental conditions, the stability of its quality depends on temperature and light effects.

Table 5 gives the results of calculations on the effect of aromatization upon the change of taste in the orange drinks. It is shown by the results that of the three differently aromatized samples, stored at 20 °C in the dark the taste of the sample aromatized with natural aroma deteriorated most rapidly. This one differed from the other two samples at the 95% probability level.

Out of the three samples stored at 20 °C exposed to light the rate of deterioration of the taste of the drink prepared from natural fruit juice was the highest and this rate differed very highly significantly at the 99% probability level from the deterioration rate of the other two samples.

Out of the three samples stored at 5 °C in the dark the rate of quality change of the sample prepared with natural aroma was the highest and it differed at the 95% probability level from that of the other two samples.

To sum up the conclusions the comparison of deterioration rates has shown that protection of the quality of the nutrition biologically more valuable natural fruit juice requires special care. From this aspect the most important is to regulate the effect of light and storage temperature. To exclude or reduce the influence of light can be solved by selecting bottles of coloured glass or some metal laminated packaging material.

The deterioration rate is increased by the change or the increase of storage temperature. The significance of the change of temperature, however, depends on the type of aromatization applied and affects the rate of change to a minor extent in comparison to light.

*

The authors wish to express their thanks for the precise and expert work of the colleagues in the industrial branch laboratories and enabled thereby the objective evaluation of the results.

Literature

BASETTE, R. (1976): Effects of light on concentration of some volatile materials in milk. *J. Milk Fd Technol., 29,* 10–12.

FARKAS, J., SPANYÁR, P. & ZUKÁL, E. (1975): "Az élelmiszerek eltarthatósági idejének meghatározása" kutatási program irányelvei és értékelésének szempontjai. (Directives and aspects of evaluation of the research programme: Determination of keeping quality of food products.) *Élelm. Ipar, 29,* 353–359.

GACULA, M. C. Jr. (1975): The design of experiments for shelf-life study. *J. Fd Sci., 40,* 399–403.

GACULA, M. C. Jr. & KUBALA, I. I. (1975): Statistical model for shelf-life failures. *J. Fd Sci., 40,* 404–409.

HUNGARIAN STANDARD (1974): Alkoholmentes üdítő italok. Érzékszervi pontozásos bírálat. (Alcohol-free soft drinks. Sensory score test.) MSZ 08–1526–74.

KAREL, M., MIZRAHI, S. & LABUZA, T. P. (1971): Computer prediction of food storage. *Mod. packag., 36,* 212–215.

KÖRMENDY, L. (1983): *Bevezetés a matematikai statisztikába.* (Introduction into the mathematical statistics.) Budapesti Műszaki Egyetem Mérnöki Továbbképző Intézet, Budapest.

LABUZA, T. P. (1980): Enthalpy/entropy compensation in food reactions. *Fd Technol., 34,* 67–78.
LABUZA, T. P. (1982): *Shelf-life dating in foods.* Food and Nutrition Press, Inc., Wesport.
LABUZA, T. P. & RIBOH, D. (1982): Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. *Fd Technol., 36,* (10), 66–73.
MAN de, F. (1978): Possibilities of prevention of light-induced quality loss of milk. *J. Inst. Can. Sci. Technol. Aliment., 11,* (3), 152–154.
MIZRAHI, S., LABUZA, T. P. & KAREL, M. (1970): Computer aided prediction of food storage stability. *J. Fd Sci., 35,* 799–813.
PRÉKOPA, A. (1971): *Valószínűségelmélet műszaki alkalmazásokkal.* (Probability theory with technical applications.) Műszaki Könyvkiadó, Budapest.
QUAST, D. G. & KAREL, M. (1972): Computer simulation of storage life of foods undergoing spoilage by two interactive mechanisms. *J. Fd Sci., 37,* 679–691.
QUAST, D. G. & KAREL, M. (1973): Simulating shelf-life. *Mod. packag., 38,* 159–165.
RIBOH, D. & LABUZA, T. P. (1982): Kinetics of thiamine losses in pasta stored in a shine wave temperature condition. *J. Fd Process. Preserv., 6,* (4), 253–259.
SIMON, L., LABUZA, T. P. & KAREL, M. (1971): Computer aided prediction of food storage stability: oxidative deterioration of a shrimp product. *J. Fd Sci., 36,* 280–285.
VARSÁNYI, I. & SOMOGYI, L. (1982): Az élelmiszerek tárolás közbeni minőségváltozásának modellezési lehetőségei. (Possibilities of modelling quality changes in foods during storage.) *Élelm. Ipar, 36,* 281–286.
VARSÁNYI, I. (1985): *Élelmiszeripari csomagolástechnika.* (Food packaging techniques.) Mezőgazdasági Kiadó, Budapest.
VARSÁNYI, I. (1987): Shelf-life prediction of food. — In: *Current trends in the food industry.* EOQC, Bern, Switzerland, pp. 55–60.

BOOK REVIEWS

Food emulsions

K. LARSSON & S. E. FRIEBERG (Eds)

Marcel Dekker, Inc., New York and Basel, 1990, 504 pages

This second edition of the book presents both fundamental and applied aspects of food emulsions — covering the *latest* progress in lipid association structures such as liquid crystals and their influence on technical processes. It contains eleven well-detailed chapters written by internationally selected authors. All the chapters are completed by a generous list of references.

The six theoretical chapters discuss emulsion stability, the influence of association structures on it, HLB system. An important development since the earlier edition is the treatment of forces at surfaces, particularly hydration forces. A main objective has been to provide a broad understanding of surface and colloid science relating to the components of food emulsions, and then to focus on the most common types of food products as milk and dairytype emulsions, dressings and sauces, ice cream, beverage emulsion, baking mechanism in cake production. The theoretical chapters are well connected with the applied chapters, which is demonstrated by the large number of interchapter references. This publication is a uniquely modern and highly important contribution to our knowledge of food emulsions. It should be of interest both to scientists and to professionals working in product development, controlling and production.

F. MOHOS

High barrier plastic films for packaging

Volume 1. Plastic film technology

KIER M. FINLAYSON (Ed)

Technomic Publishing Co. Inc. Lancaster, Basel, 1989, 266 pages

Plastic film packaging is an area of current growth, improvement and innovation. A compilation of twenty-three technical reports presents the technology behind these advances in plastic film packaging materials.

These reports, selected from the *Journal of Plastic Film and Sheeting*, provide in-depth information on film resins, plastic films and film constructions, film-making processes, test methods and performance data, and film use in different types of packages and with different types of products.

This collection of reports is mainly in direct contact with food packaging. Several papers deal with: barrier behaviour of food packaging; the correlation between the permeable packages and the shelf life of foods; the materials that may be used for aseptic packaging; the possibilities of improving the barrier behaviour of packaging materials; retortable plastic packaging materials; correlations between processing and production and the barrier behaviour of plastic foils. The references after each chapter provide great help to those who wish to deal with the certain problem in depth.

The thematics and the content make the book useful in both industrial practice and in research work. The theoretical considerations and statements in the reports highly contribute to education, too.

I. VARSÁNYI

Akadémiai Kiadó, Budapest

Biosensors International Workshop 1987

GBF Monographs, vol. 10.

R. D. SCHMID, G. G. GUILBAULT, I. KARUBE, H. L. SCHMIDT & L. B. WINGARD (Eds)

VCH-Verlagsgesellschaft, Weinheim, 1987, 346 pages

In the last years the tremendous improvement in signal transducer technology promoted the coming in the general use of the biosensors, pushing the advantages in the manufacturing of microelectrodes, FETs, fiber optics and piezocrystals. Leading researchers from 16 countries took part on the GBF Workshop in Braunschweig (GDR) from June 23–26, 1987. It was considered by most participants that it has provided a practically complete overview of the state-of-the-art in the field of biosensors. In this monography the topics of the delivered lectures and exhibited posters are summarized.

The English publication contains the summary of 34 lectures and 37 posters. The chapters of this book in accordance with the sections are the following: Electrode Probes, Flow Injection Analysis, New Sensors/Immunoprobes, Transistor Devices, Piezo/Optical Sensors and "Horizon Lectures".

In the first section Rechnitz sketched the future of biosensors for the 1990's. They may play an important role in sensing and controlling because of their simplicity and cheapness. The authors reported about a lot of practical methods/implementations. They prepared amperometric glucose sensor for meat freshness, glucose sensor for use in fermentation systems, sensor for detecting aromatic amines.

In clinical practice the glucose and its metabolites are measurable in blood, biosensors were applied to estimate L-glutamine, glucose and L-lactate concentrations in control of a process involving mammalian cell culture. Direct and indirect electron transfers to redox proteins and enzymes were studied by using electrochemical methods.

The lectures in the second part deal with flow injection analysis. Ruzicka reported on the development of the electrochemical, fibre optic and piezoelectric biosensors being used for labor and process controlling purposes, and showed their operation by an example of measuring urea and pH. There was a report on a flow system with immobilized dehydrogenases for simultaneous monitoring of glucose and ethanol during a baker's yeast fermentation.

In the third section enzyme immunoassay was studied with electrochemical detection in such a way that the antigen had been labelled with an enzyme for catalyzing the production of an electrochemically detectable product. Biosensors based on neuroreceptors were presented with the example of an acetylcholine receptor and gamma-amino butyric acid receptor. An account was given of researches on biological membranes and how they could serve as the base of a new class of chemical sensors with the help of added highly selective receptors.

In the fourth section the new biosensor systems were studied with the help of the newly developed field effect transistors. The base of the micro device introduced by Karube was amorphous silicone ion field effect transistor (a-ISFET) used as a pH-sensor.

Fish freshness sensor, micro-glucose sensor, micro-glutamate sensor and micro-oxygen sensor were constructed by immobilizing several enzymes on the surface of the signal transducer. There were some studies on ISFETs and REFETs covered by enzymes as well as on the enzyme modified and immuno-selective ISFETs.

In the fifth section the piezo and optical sensor-based biosensors were presented. For example, Guilbault prepared protein coated piezoelectric crystals for the assay of gaseous pollutants. Fluorescent indicator covered optochemical sensors (optodes) were used for continuous monitoring of blood gases as well as measurement of pH and ionic strength. Luminescent immunosensors and electrochemical luminescent immunosensors had been developed to determine small molecules such as hormones and pharmaceutical drugs. The role of fiber optic biosensors and the possibility of introducing fiber optic measuring cells into biotechnology was studied.

In the sixth part the "horizon lectures" were delivered. Ulmer reviewed a new generation of biosensors based on the rapidly developing field of protein engineering. Problems which have previously limited the application of biosensors, such as stability

and immobilization efficiency are likely to be overcome by the changing of the three-dimensional atomic structure of protein, enzymes and antibodies.

Nakajima spoke about the Human Frontier Science Program, how the development of biotechnology aims at elucidating the essential functions and mechanism of human bodies and biobodies, for example the brain functions and the energy exchange functions. Oesterhelt reviewed the properties of bacterial rhodopsins, considering that bacteriarhodospin can be used as a light-driven proton pump and halorhodopsin as a light-driven chloride pump.

Summing up this monography it can be admitted that it gives a wide up-to-date report on the possibilities of using biosensors in research and in the practice.

NORA ADÁNYI

The chemistry and technology of edible oils and fats and their high fat products

G. HOFFMANN (Ed)

Academic Press Limited, London, 1989, 384 pages

Edible oils, fats and their high fat products are world-wide important and indispensable foods.

Several people are involved in their production and in the development of production, and therefore more and more information are available.

The author obtained more than 30 years experience in this field, mostly from the Unilever Research Laboratory. His special merit is that synthetizing the publications of this field he made a compact and nevertheless clearly understandable book available. The publication gives a comprehensive introduction on the subject, deals with its up-to-date situation and with the modern methods of producing various products. The book has 8 chapters.

So as to understand the subject the first chapter deals with the condense description of the major lipid components and with fat modifying reactions.

The following two chapters discuss the most essential fat separation methods from fruit pulps and seeds and from animal "fatty tissues" with special emphasis on introducing the most modern methods.

Logically, the following chapters deal with the refining, then a longer one with the most important fat modification processes (hydrogenation, fractionation, interesterification) which are essential for the understanding of the production, characteristics and the modes of application of high fat products — like margarines, shortenings, mayonnaise, special fats — which are discussed very comprehensively and in detail in the following two chapters.

Specially important is that the book examines the principle background of every necessary process and so enabling the explanation of practical methods.

The book mentions the history of the most important operations, phases of development and enables the understanding of the advantages of modern methods.

The last chapter contains important practical data about storage, transportation and energy requirement.

The valuable monography is carefully written. It provides useful information for experts dealing scientifically and in practice with edible fats and with fat like products. The book may widely be recommended for study.

J. PERÉDI

Le magnésium en pratique clinique

JEAN DURLACH (Ed)

J. B. Bailliére, Editions Medicales Internationales, Paris, 1985, 387 pages

The original French edition of Jean Durlach's book *Le Magnésium en pratique clinique* published by J. B. Balliére Editions Médicales Internationales, Paris in 1985 was translated into English by David Wilson and published by John Libbey Company Ltd. London in 1988, an event which by itself emphasizes the value of the book.

I would begin by remarking that the title of the book does not cover its content entirely. An important, exhaustive and thoroughly documented part of the book presents the scientific research carried out in this field, especially in the last three decades.

After this period of intense research work in the field of Mg deficiency in human pathology, the value of the results asked for the creation of an international forum of communication realized by J. Durlach which evidenced his exceptional qualities of scientific organizer. The First International Symposium of Magnesium (I-er Symposium International sur le déficit magnésique en pathologie humaine 1971) was followed by other 4 international symposia on the same theme. The last symposium held in Kyoto in 1988 ensured the communication of the most recent information in the field. The English version of the book *Le Magnésium en pratique clinique — Magnesium in clinical practice* — was presented at this symposium.

The book, conceived as a logical systematization of the results obtained, their application in medical practice and biology, as a confrontation of ideas and even contradictory data, documented by a complete list of references up to 1984, is a real breviary of magnesium placed at the disposal of research workers and clinicians. The book also documents the incontestable value of this bivalent ion, its importance in the adaptation of the superior living organism to physical and physical stress, radiobiological stress, biological stress represented by diseases of the most various etiology, environmental stress.

The fundamental conclusion of the book is to be found on p. 74 of the English edition (p. 84 in the French one): "Our diet is marginal in magnesium, and we live with a chronic deficiency." A conclusion having the value of an important discovery in medicine, which tells us that under the present living conditions a magnesium deficiency restored by an adequate substitution therapy may appear once again, and even more than once.

J. SZÁNTAY

Production and packaging of non-carbonated fruit juices and fruit beverages

D. HICKS (Ed)

Blackie and Son Ltd, Bishopbriggs, Glasgow and London, 1990, 416 pages

In this book an international team of specialists review the fruit juice and fruit beverage industry, from grower to distributor, including fruit handling and processing, chemistry and characterisation, analysis, quality control, nutritional value and packaging.

The book is introduced by a preface which is followed by the list of contributors and the content. The 14 chapters are followed by a glossary and an index. The book is very well illustrated, adequate number of figures, tables and photos help to study the subject in full details.

Out of the 14 chapters, 10 discusses the chemistry and technology of various fruit juices. Closely connected to these are the chapters about nutritional value and safety; about packaging systems; legislation controlling production; labelling and marketing. Production, consumption and flavour references are discussed in the last chapter. Each chapter is supplemented by references to get more information on the subject.

This volume is written for food scientists and technologists, food processors and packaging engineers, and will be a source of reference for researchers and academics within these disciplines.

I. VARSÁNYI

Formulation and production of carbonated soft drinks

A. J. MITCHELL (Ed.)

Blackie and Son Ltd., Glasgow, 1990, 361 pages

The book deals with carbonated soft drinks' production and development through the consumption in the USA, Europe and Japan. Marketing conditions in certain states and countries are dealt with and also the distribution of well-known alcohol-free soft drinks are given in percentage.

Each chapter may be considered an individual study. Water treatment chapter is about analyses from hard and soft areas; effects of impurities in water; coagulation, ion exchange, reverse osmosis, alternative sterilization methods and future developments.

Carbohydrate sugars chapter deals with granulated sugars, starch derivatives; glycose syrups, high-fructose syrups, granulates and with the ways of utilization, transportation and storage.

The high-intensity sweeteners chapter discusses saccharin, cyclamate, acesulfame K, aspartame, stevioside, thaumatin, dihydrochalcones and their utilization.

Flavourings and emulsions chapter distinquishes 3 categories of flavourings as follows: natural flavourings, nature identical flavourings and artificial flavourings. Oil phase and water phase emulsions are discussed separately.

Acids, colours, preservatives and other additives chapter discusses the additives used depending on the character of the soft drink in 5 tables. The safety of food additives and the ADI values are also dealt with.

Syrup room operations chapter discusses the syrup treatment in details. Development tends towards computerized treatment. The Clean-in-Place (CIP) system is shown which includes sterilization.

Containers and closures chapter includes the various traditional and newly developed packaging materials and means, their consistency, character and also filling and closing operations.

Handling empty containers chapter provides detailed handling instructions and also describes the necessary equipments.

Carbonation and filling chapter mentions the properties of carbohydrate, its measurement and determination. The steps of filling are illustrated in figures and with suitable machines.

Container decoration chapter discusses the main types of decoration application machinery and the special container decoration applications and systems.

The book devotes a separate chapter on container-inspection equipment and another on secondary and tertiary packaging.

Effective application of quality control chapter reviews the evolution and growing importance of quality control and quality assurance in the soft drink industry.

The book may arouse the interest of those dealing with beverage containers. An excellent handbook of soft drink technologists, chemists, packaging experts regarding either the beginners or the experienced experts who wish to specialize further in a particular field. Personnel involved in distribution, sales, marketing and financial matters inside the soft drink industry will also gain a valuable help.

É. SZÁNTÓ-NÉMETH

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Investigation of aqueous solutions of sucrose, D-glucose and D-fructose with positron lifetime spectroscopy
K. Süvegh, F. Mohos & A. Vértes

Effect of home preparative procedures and technological processes on lindane residues in tomato
B.A.A. Bessar, K. Korány & A.S. Szabó

Free radical reactions in meats
E. Dworschák, A. Lugasi, A. Blázovics, GY. Biró, P. Biacs & Á.J. Zsinka

Influence of the state of ripeness of Chardonnay grapes on wine composition. — Part I. Physicochemical characteristics, higher alcohols, polyols and esters
M.P. Callao, J.M. Borras, A. Lopez & F.X. Rius

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 19 No. 4 — 1990

CONTENTS

Index: 26.039